Introduction to Cheminformatics

Cheminformatics is a field of information technology that uses computers and computer programs to facilitate the collection, storage, analysis, and manipulation of large quantities of chemical data. Chemical data includes chemical formulas, chemical structures, chemical properties, chemical spectra, and biochemical or biological activities. The term cheminformatics, which is an abbreviated form of "chemical informatics," was first coined by Frank Brown about 10 years ago (Brown, 1998). However, the central concepts behind cheminformatics, such as quantitative structure–activity relationships (QSARs) and compound property prediction, have been around for more than 30 years. Until recently cheminformatics was a relatively obscure discipline with a comparatively small academic or industrial presence. However, with the advent of high throughput drug screening and the need for million-compound chemical libraries, cheminformatics is now playing a key role in many aspects of drug discovery and drug development. Cheminformatics is also playing a vital role in emerging fields such as chemical genomics (Yang et al., 2006), systems biology (Schnackenberg and Beger, 2006), and metabolomics (Schlotterbeck et al., 2006; Wishart et al., 2006). Indeed, as shall be seen shortly, cheminformatics has much to offer to the fields of molecular biology, biochemistry, and bioinformatics.

Cheminformatics (as it is known in North America), or chemoinformatics as it is known in Europe and the rest of the world, is actually a close cousin to bioinformatics. However, the two fields have largely evolved along separate, almost divergent paths. For instance, many cheminformatics resources are expensive, closed source (i.e., precompiled), and distributed through commercial vendors. In contrast, most bioinformatics resources are free, open source, and distributed through the Web. This difference reflects the fact that the field of chemical informatics started in the 1970s. During this era the standard model for software or database distribution was through commercial entities and the primary clients were multinational drug companies. On the other hand, most bioinformatics software emerged much later (in the 1990s) and the field was heavily influenced by the open source movement, the emergence of the Web (for distribution) and the fact that most clients were academics.

The differences between cheminformatics and bioinformatics are also reflected in their database content. Many chemical compound databases were developed without the expectation that this information might eventually be biologically or medically relevant. As a result most chemical data is (still) not linked in any meaningful way to biological data such as protein targets or their downstream physiological effects. Likewise, most bioinformatics databases were developed without the intention of using this data to facilitate drug or drug-target discovery. Consequently most sequence data is not linked in any meaningful way to existing drug or disease information. This lack of data overlap in database content has led to cheminformatics and bioinformatics drifting uncomfortably far apart.

However, thanks to a number of new funding initiatives (such as the NIH Roadmap initiative) along with the coincidental emergence of chemical genomics, systems biology, and metabolomics, there is now a growing desire to bring bioinformatics and cheminformatics closer together. This has led to an increasing number of freely available, open-source or Web-enabled databases and software tools. A number of these public tools will be discussed in detail in this cheminformatics unit, including Pharmabase (*http://www.pharmabase.org*), MSDchem (Golovin et al., 2005), DrugBank (Wishart et al., 2006), ZINC (Irwin and Shoichet, 2005), and others. Many other freely available resources will also be briefly reviewed in this short introduction to the field of cheminformatics. These open source, Web-enabled tools are now making cheminformatics far more accessible and far more relevant to biologists, medicinal chemists, and bioinformaticians (Geldenhuys et al., 2006).

THE INTERSECTION BETWEEN CHEMINFORMATICS AND BIOINFORMATICS

A partial comparison between the types of databases and software found in both cheminformatics and bioinformatics is given in Table 14.1.1. As seen in this table, there are actually a remarkable number of similarities between

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Table 14.1.1 Comparisons Between Databases, Data Formats, Prediction Methods, Visualization Software and Manipulation Tools Used in Bioinformatics and Cheminformatics

Bioinformatics		Cheminformatics	
Type	Name	Type	Name
Archival sequence databases	GenBank	Archival compound databases	PubChem
Curated databases	SwissProt, UniProt, RefSeq, FlyBase, SGD, HPRD	Curated databases	ChEBI, KEGG (*UNIT 1.12*), DrugBank (*UNIT 14.4*), PharmaBase (*UNIT 14.2*), HMDB
Pathway databases	Reactome (*UNIT 8.7*), BioCarta, KEGG (*UNIT 1.12*)	Pathway databases	KEGG (*UNIT 1.12*), MetaCyc, PharmGKB
Structural databases	PDB (*UNIT 1.9*), MSD (*UNIT 14.3*)	Structural databases	ZINC, Ligand Depot (*UNIT 1.9*)
Sequence string format	FASTA (*APPENDIX 1B*)	Chemical string format	SMILES, InCHI
Data exchange format	BioXML, BSML	Data exchange format	CML
Format conversion software	Readseq (*APPENDIX 1E*)	Format conversion software	OpenBabel
Structure format	PDB (*UNIT 1.9*), mCIF	Structure format	MOL, SDF, PDB (*UNIT 1.9*)
Sequence similarity searching	BLAST (*UNITS 3.3 & 3.4*), Needleman-Wunsch	Chemical similarity searching	Tanimoto Algorithm, Subgraph Isomorphism
Gene identification software	GenScan	Chemical identification software	ChekMol
Property prediction	Hydrophobicity	Property prediction	LogP
Property prediction	pI	Property prediction	pKa
Property prediction	Solubility	Property prediction	Solubility
Property prediction	Molecular Weight	Property prediction	Molecular Weight
Protein/peptide ID software	Mascot, Aldente, Phenyx	Chemical ID software	NIST/EPA/NIH Mass Spec Library, SDBS, AMDIS
2-D structure prediction software	PsiPred	2-D structure prediction software	MolConvert
3-D structure prediction software	Rosetta	3-D structure prediction software	Corina
Structure visualization applet	QuickPDB, JMol, WebMol	Structure visualization applet	Chime, JME
Ontology	Gene Ontology	Ontology	ChEBI Ontology
Protein-protein interaction prediction	PIPE, TSEMA	Protein-ligand interaction prediction	Glide (*UNIT 8.11*), GOLD; FlexX; Dock

cheminformatics and bioinformatics. For instance, both have a central need for electronically accessible databases. Typically bioinformatics databases consist of large collections of protein or DNA sequences and/or structures, while cheminformatics databases consist of large collections of chemical formulas, names, and structures. It is also evident that both cheminformatics and bioinformatics have a critical need for database search tools with bioinformatics needing sequence and structure searching software and cheminformatics needing software to match molecular substructures or SMILES strings (Weininger, 1988). The similarities extend even further with both disciplines requiring: (1) data exchange standards; (2) standardized names, vocabularies, or ontologies; (3) structure visualization

software; (4) compound (MS) identification tools; and (5) property prediction software. Obviously, in bioinformatics the focus is on large molecules (proteins, DNA, and RNA) while in cheminformatics, the focus is on small molecules (<1000 Da).

The linkage between small molecules and large molecules is what ultimately connects bioinformatics with cheminformatics. After all, large molecules such as proteins, RNA, and DNA are composed of small molecule constituents (amino acids and nucleotides). Not only is there a constitutive relationship, but there is a functional relationship as well. Small molecules act on large molecules and vice versa. For instance, most small-molecule drugs (of which 99% are small molecule compounds) act on large-molecule protein or DNA targets. Vitamins, metal ions, and other small molecule cofactors regulate the function and activity of most proteins and many genes. Likewise, large molecules such as genes and proteins are ultimately responsible for mediating the synthesis and degradation of most small molecules (drugs, nutrients, and metabolites). The connection between small molecules and large molecules extends throughout almost all cellular processes. Indeed, the interplay between small molecules (the environment) and large molecules (the genotype) is what fundamentally defines an organism's phenotype.

To make the connections between bioinformatics and cheminformatics a little clearer it is perhaps useful to briefly review some of the key software resources now used in cheminformatics. In particular, three main software categories will be covered including: (1) cheminformatic databases; (2) database searching tools; and (3) property prediction tools. Also highlighted in this unit is how the tools or databases in each of these categories are making, or can make, the vital connections to biology and bioinformatics.

DATABASES IN CHEMINFORMATICS

There are three types of cheminformatic databases: (1) archival or "global" compound databases; (2) specialized or highly curated databases; and (3) structural databases. This closely parallels the situation in bioinformatics where there are archival or global sequence databases like GenBank (Wheeler et al., 2006), specialized sequence databases like GeneCards (Rebhan et al., 1998), HPRD (Mishra et al., 2006), or SwissProt (O'Donovan et al., 2002; Bairoch et al., 2005) and structural databases such as the Protein Databank (PDB; Westbrook et al., 2002) or MSD (Brooksbank et al., 2005). In contrast to most bioinformatics databases, which are almost all free, the majority of cheminformatics are commercial. However, there are also a growing number of high quality, freely available cheminformatic databases.

The largest publicly accessible database of chemical information is PubChem (Wheeler et al., 2006). PubChem is supported by the NIH's Molecular Libraries Roadmap Initiative, so it is mandated to provide information about small molecules and the biological activities of as many small molecules as possible. PubChem (PC) includes substance information, compound structures, and bioactivity data in three primary databases: PC-Substance, PC-Compound, and PC-BioAssay. Like GenBank, PubChem was developed and maintained by the National Center for Biotechnology Information (NCBI). Strictly speaking PubChem is an archival database, as it contains data deposited by many different organizations, labs, and companies (50+ at last count). Currently, PubChem contains more than 10 million unique compounds, each of which have chemical structure information, common names, IUPAC names, SMILES strings, InChI identifiers, molecular weights, chemical formulas, LogPs, and other compound descriptors. PubChem is extensively linked to PubMed and many compounds have descriptions of their biological activity provided through PubMed abstracts. Because of its size, its accessibility, and its high standards, PubChem has become the GenBank of the cheminformatics world.

In the second category of databases (curated or highly annotated) are a number of smaller, more specialized resources. A partial list of these databases includes KEGG (Kanehisa et al., 2006), MetaCyc (Caspi et al., 2006), DrugBank (Wishart et al., 2006), Pharmabase (*http://www.pharmabase.org*), TTD (Chen et al., 2002), HMDB (Wishart et al., 2007), ChEBI (Brooksbank et al., 2005), and PharmGKB (Hewett et al., 2002). Rather than containing millions of compounds, these databases typically contain thousands or tens of thousands of bioactive compounds. What distinguishes these databases from PubChem is the fact that they include detailed information describing not only bioactive small molecules but also their associated biological pathways, macromolecular targets,

mechanisms of action, biological effects, disease associations, toxicological data, and pharmacogenomic consequences. Most of these databases also have extensive search and browsing capabilities, including text, sequence, and structure similarity searches.

The third category of cheminformatic databases are structural databases containing 3-D coordinate data. Some databases, such as the Cambridge Structure Database (*http://www.ccdc.cam.ac.uk*) contain the 3-D coordinates of chemical structures that were determined experimentally. The Cambridge Structure Database (CSD) is the chemical analog of the Protein Data Bank (Westbrook et al., 2002). However, unlike the situation with macromolecules, where ab initio 3-D structure prediction is still an unsolved problem, the 3-D structure of most small molecules can be accurately predicted from their 2-D structures or SMILES strings (Sadowski and Gasteiger, 1993). In fact, there are a number of freely available programs and Web servers such as MolConverter (ChemAxon), CORINA (Sadowski and Gasteiger, 1993), CACTVS (Ihlenfeldt et al., 2002), or the Cactus online Converter (*http://cactus.nci.nih.gov/services/translate/*), that can take stick figure diagrams (MOL and SDF files) or SMILES strings and generate high-quality 3-D coordinates in PDB file format. As a consequence, most of today's 3-D coordinate databases contain predicted 3-D structures rather than experimentally determined structures. These 3-D databases are particularly useful for virtual screening efforts where large libraries of compounds are rapidly docked onto a known protein structure using such ligand docking programs as Dock (Shoichet and Kuntz, 1993), FlexX (Kramer et al., 1997), or Glide (Halgren et al., 2004).

Some examples of these 3-D databases include ZINC (Irwin and Shoichet, 2005), Ligand Depot (Feng et al., 2004), and the NCI 3-D Structure Database (Milne et al., 1994). ZINC, which is a recursive acronym for "Zinc Is Not Commercial", is a database containing modeled 3-D structures of nearly 4.7 million commercially available small molecules. To facilitate docking or drug discovery studies, each of the compounds are assigned biologically relevant protonation states. They are also annotated with relevant physical properties such as molecular weight, LogP, and number of rotatable bonds. Every molecule in ZINC contains vendor information and is ready for "virtual screening" using most of the common molecular docking programs. ZINC supports several common file formats including SMILES, mol2, 3-D SDF, and DOCK format. A Web-based query tool incorporating a molecular drawing applet allows the database to be searched and a variety of structure subsets to be created.

The National Cancer Institute (NCI) Drug Information System (DIS) 3-D database is a collection of modeled structures for over 400,000 primarily organic compounds which have been tested by NCI for anticancer activity. The NCI 3-D or NCI Open database is maintained by the NCI's Developmental Therapeutics Program. The database is actually an extension of the NCI Drug Information System. Recent comparisons to common commercial databases suggest that the NCI-3D database has by far the highest number of unique compounds. Approximately 200,000 of the NCI structures were not found in any of the other analyzed databases (Voigt et al., 2001). The actual structural information stored in the NCI-3D database is the connection table for each compound, which is just a list of which atoms are physically connected and how they are connected. Connection tables provide sufficient information to generate accurate 2-D and 3-D structures, as well as unambiguous SMILES strings. As a result, several variations of the NCI-3D database have been prepared using various format conversion and structure generation tools (see *http://cactus.nci.nih.gov/ncidb2/download.html*). These freely available files can be used to set up local databases that can be used for docking and virtual screening. NCI-3D can also be searched using compound similarity searching tools (see next section) to find similar compounds having comparable biological activity.

Unlike ZINC or the NCI-3D database, the Ligand Depot is a database that contains actual structural coordinate data. While many times smaller than ZINC, NCI-3D, or even the Cambridge Structure Database, what makes the Ligand Depot particularly appealing is the fact that it contains structures of small molecule compounds bound to protein or DNA targets. As a result, the structural information in Ligand Depot is highly relevant to docking studies. Furthermore, the information contained in Ligand Depot can be used to train docking software or it may be used in predicting or determining optimal conformers in 3-D structure prediction programs. So, while Ligand Depot is not routinely used as a compound database for virtual screening, it is used to facilitate the creation of compound databases and the optimization of many docking software packages.

DATABASE SEARCHING IN CHEMINFORMATICS

In the world of informatics, databases are relatively useless if they cannot be easily searched. Obviously searching for exact string or numeric matches is relatively trivial, but in both bioinformatics and cheminformatics, there is a central need to perform "fuzzy" or inexact matching. In other words, researchers want to find approximate matches to their query sequences or structures. In conventional bioinformatics, database sequence (or string) matching and searching is done using dynamic programming (Needleman and Wunsch, 1970) or heuristic search programs like BLAST (Altschul et al., 1997). In structural bioinformatics, structure searching is done through structure superposition or substructure matching tools such as DALI (Dietmann et al., 2001), CE (Shindyalov and Bourne, 2001), and VAST (Gibrat et al., 1996).

In cheminformatics, there are a number of equivalent methods to perform both "sequence" (i.e., string) and structure matching against large chemical compound libraries. Thanks to the development of standardized text representations of chemical compounds through InChI (IUPAC International Chemical Identifier) strings and SMILES strings, it is possible to give every chemical a unique character string. In other words, InChI and SMILES strings uniquely define chemical compounds, much like a gene or protein can be uniquely defined by its sequence. As a result, if a chemical database such as PubChem, ZINC, or DrugBank is converted into a collection of SMILES strings or InChI identifiers, it is then possible to use character string comparison to do compound matching. Several Web-based conversion sites, including the Molecular Structure File Converter (*http://iris12.colby.edu/~www/sconv.cgi*), the Cactus Structure File Converter (*http://cactus.nci.nih.gov/services/translate/*), and the InChI converter (*http://inchi.info/converter_en.html*) are now available to facilitate conversion between MOL, SDF, PDB, SMILES, and InChI formats.

The actual string or "sequence" search algorithm requires that both the query compound and the database of searchable compounds be expressed in SMILES or InChI strings. The algorithm uses common string parsing and string matching utilities, similar to those found in spell-checking software, to score the similarity between the query character string and the database character strings. Unfortunately, this approach is not always fool-proof. The scoring schemes for chemical substring matching are not yet as sophisticated as they are with sequence matching algorithms. Likewise, there are several different SMILES string dialects, which makes it difficult to exchange databases or search algorithms.

More sophisticated chemical structure matching algorithms also exist. These are based on the idea of matching substructures. However, because the structures of chemical compounds are far more diverse than what is seen for proteins, the structure matching utilities in chemistry have to be slightly more sophisticated. In particular, chemists must use the concept of subgraph isomorphisms (Ullman, 1976) and adjacency matrices to identify chemical similarity. For substructure searching, the 2-D chemical structures of both the query and database compounds must be rewritten as tables that indicate the bond connectivity between each pair of atoms. These tables, which have 1s for connected atoms and 0s for unconnected atoms, are called adjacency matrices. The name comes from the fact that they indicate which atoms are adjacent (connected) to each other. Once prepared, the adjacency matrix from the query structure is compared to every adjacency matrix in the database. If substantial sections of the query matrix match to an adjacency matrix (or portion thereof) in the database, then it is likely that the two structures are similar. Different scoring schemes and adjustable threshold cutoffs may be used to distinguish strong matches from weak matches or to identify compounds with particularly important substructures.

PROPERTY PREDICTION IN CHEMINFORMATICS

Compound property prediction is something common to both bioinformatics and cheminformatics software. In bioinformatics, the compounds being analyzed are typically macromolecules such as peptides, proteins, RNA, or DNA. In cheminformatics, the compounds being analyzed are usually small molecule drugs, drug leads, toxins, or metabolites. In bioinformatics, the properties of interest include hydrophobicity, isoelectric point, UV absorbance, molecular weight, flexibility, secondary structure, radius of gyration, stability, and solubility. In cheminformatics, the properties of interest include electronic or charge distribution, preferred conformations, heats of formation, solubility, LogP, pK_a, refractivity, melting point, molecule length, molecular area, molecular volume, and

reactive groups. Some of these chemical properties, such as solubility, LogP, and charge are particularly relevant to understanding or predicting the activity, absorption, distribution, and metabolism (ADMET) of drug compounds (Hansch and Zhang, 1993; Hou and Xu, 2003).

Chemical property prediction has been an integral part of cheminformatics software for more than 30 years. Like bioinformatics, most of the techniques used in cheminformatics property prediction make use of such machine learning techniques as artificial neural networks, decision trees, hidden Markov models, and support vector machines. Cheminformatic prediction methods also use more conventional techniques, such as hierarchical clustering, principal component analysis, and correlational analysis. Most of today's commercial chemistry software vendors, such as ACD labs, CambridgeSoft, Tripos, and Accelrys offer at least some kind of chemical property prediction software. However, many of these predictions are also freely available over the internet through a variety of Web servers (Van de Waterbeemd and De Groot, 2002; Tetko, 2003). Examples of two simple property prediction servers include the Actelion Property Explorer and Pre-ADMET.

The Actelion Property Explorer (Google "Actelion Property Explorer") is a Web-enabled Java applet that allows users to draw chemical structures and then rapidly calculate various drug-related properties, including toxicity risks (mutagenicity, tumorgenicity, irritancy, and reproductive effect), solubility, logP, molecular weight, drug-likeness, and overall drug score. Like the Actelion server, Pre-ADMET (*http://preadmet.bmdrc.org/preadmet/index.php*) offers a wide range of ADME and toxicological property calculations for any submitted chemical compound. Three classes of predictors are supported, a molecular descriptors calculation, a drug likeness predictor, and an ADME predictor. The molecular descriptor calculator can predict nearly 1000 molecular properties including constitutional, topological, physicochemical, and geometrical descriptors, many of which are needed for ADMET prediction. The drug likeness predictor is very simple and uses Lipinski's rules (*Rule of Five*) and lead-like rules in its predictions. The ADMET predictor is quite unique and can predict permeability for Caco-2 cells, MDCK cells and BBB (blood-brain-barrier), HIA (human intestinal absorption), plasma protein binding, and skin permeability using an artificial neural network. Users can draw input structures using a simple structure drawing applet or upload compound files in "sdf" or "mol" file format.

CONCLUSION

As emphasized throughout this chapter, cheminformatics and bioinformatics are rapidly evolving disciplines in information technology that share many common features. Both fields need databases (sequence and structure databases in bioinformatics; structure/activity databases in cheminformatics), both fields depend critically on database searches and comparisons (sequence and structure comparison in bioinformatics; structure comparison in cheminformatics), and both fields focus on making predictions using modern pattern recognition and data mining techniques. The fundamental difference between cheminformatics and bioinformatics lies in the size of the molecules that they study. In cheminformatics the molecules are typically $<$1000 Da, while in bioinformatics the molecules are typically $>$10,000 Da. These size differences lead to some fairly fundamental differences in what is predictable, what is searchable, and what is observable. Nevertheless, as our understanding of both chemistry and biology improves, it is likely that these molecular size differences will prove to be less of a barrier to convergence than once thought. Furthermore, as the fields of drug discovery, systems biology, chemical genomics, and metabolomics become progressively more popular and progressively more computerized, it is not hard to imagine that, someday, the complete integration of cheminformatics with bioinformatics will be seen.

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A very current and very readable review of open-source software and databases, with a special emphasis on their applications to drug discovery.

Wishart, D.S. 2005. Bioinformatics in drug development and assessment. *Drug Metab. Rev.* 37:279-310.

This review touches on a number of the topics introduced in this section in somewhat more detail. The focus is more on predicting drug metabolism and drug toxicology. It is a good complement to the Jonsdottir et al. (2005) paper.

INTERNET RESOURCES

http://www.pharmabase.org

Pharmabase is a cellular physiology and pharmacology database.

http://www.ccdc.cam.ac.uk

The Cambridge Structure Database contains the 3-D coordinates of chemical structures that have been experimentally determined.

http://cactus.nci.nih.gov/services/translate/

Cactus online Converter can take stick figure diagrams (MOL and SDF files) or SMILES strings and generate high quality 3-D coordinates in PDB file format.

http://cactus.nci.nih.gov/ncidb2/download.html

Web site containing downloadable structure files of NCI Open database compounds.

http://iris12.colby.edu/∼www/sconv.cgi

Web site for Molecular Structure File Converter, which facilitates conversion between MOL, SDF, PDB, SMILES, and InChI formats.

http://cactus.nci.nih.gov/services/translate/

Cactus Structure File Converter, which facilitates conversion between MOL, SDF, PDB, SMILES, and InChI formats.

http://inchi.info/converter_en.html
InChI converter, used to facilitate conversion between MOL, SDF, PDB, SMILES, and InChI formats.

http://www.actelion.com/uninet/www/www_main_p.nsf/Content/Technologies+Property+Explorer
Web site for the Actelion Property explorer a Web-enabled Java applet that allows users to draw chemical structures and then rapidly calculate various drug-related properties.

http://preadmet.bmdrc.org/preadmet/index.php
Web site for Pre-ADMET, which offers a wide range of ADME and toxicological property calculations for any submitted chemical compound.

Contributed by David S. Wishart
University of Alberta
Edmonton, Canada

Using *Pharmabase* to Perform Pharmacological Analyses of Cell Function

In this post-genomic period of biological research, the emphasis in cell biology is returning to an understanding of cell function and dynamics, particularly with regard to protein composition and function. New technologies abound, offering methods to examine protein-dependent processes in living systems, frequently in real time. Experimentally, one popular tool for defining and manipulating such activities is the use of pharmacological compounds to alter the performance of proteins within the living cell. However, using these compounds can be a daunting process, particularly to the uninitiated. Locating the correct compound, understanding its target specificity, and even knowing how to handle and prepare it for use, are frequently in the domain of the specialist. *Pharmabase* sets out to overcome these barriers by providing simple protocols, guiding the user through a series of choices, to a database of compound records addressing the points above.

Pharmabase is a database containing detailed information on the physicochemical properties of ~1000 pharmacologically active small molecules and compounds. The compound data are linked to the target molecules, frequently proteins, organized to display their function within a cell. For example, the database is organized so that the user can navigate to known interactions between these small molecules and their receptors within the biological system of membrane transport.

This unit describes how to search and access the information in *Pharmabase*. The different search routes presented are based broadly on subject and/or graphic navigation. Getting started with *Pharmabase* and performing simple searches via subject or compound is described in Basic Protocol 1. The main way to search *Pharmabase* is via Membrane Transport (Basic Protocol 2). This subject navigator allows the investigator to access compounds targeting membrane transporters of ions and molecules. Transporters, in the context of this database, encompass channels, pumps, and porters (symporters, uniporters, and antiporters). (Further material on the diversity of these mechanisms and links to gene sites can be found at *http://www.tcdb.org* and the role of these molecules in disease can be accessed through *http://www.channelopathies.org*.; also see Rose and Griggs, 2001.) Four other subject-based navigators, derived by subset organization, are described in Basic Protocol 3. These are: Metabolism, Intracellular Messengers, Cell Signaling, and Cell Area. The compound database is sorted according to these subsets and their constitutive components. These secondary navigation routes are in place to provide a cross-referencing structure to other indexing methods and all share the purpose of reducing the database to a smaller subset of compounds and targets, tailored to the user's interest. (These navigators will be further expanded and and additional search routines based around Diseases and Tissues, as well as Action Terms, e.g., "ionophore" or "reporter" are under construction). Basic Protocol 4 describes the most recent addition to *Pharmabase*: the Graphics Navigator. Unlike the hierarchical approaches encompassed in the subject navigators described above, this protocol is relational. Target molecules are placed within cell types and pathways such that a graphic presentation and selection system allows the user to see which molecule is associated with others within the context of cell function. Currently, the graphics interface explores the insulin-secreting β-cell within the pancreas and related pathways, and expansion to other systems is planned.

Contributed by Peter J. S. Smith and David Remsen
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The focus of this database is primarily eukaryotic, multicellular animals. Expansion to other eukaryotes and prokaryotes is planned. All sections of *Pharmabase* remain works in progress as the database expands in links and content.

BASIC PROTOCOL 1

NAVIGATING THE HOME PAGE OF *PHARMABASE* USING COMPOUND AND SUBJECT SEARCH

Pharmabase allows the user to presort the database into a reduced list of subjects and compounds more tailored to the users interest. The Home Page provides the most direct access to the database, affording the capacity to search directly by individual compound or subject name.

Necessary Resources

Hardware

Computer with Internet access

Software

Web browser, e.g., MS Internet Explorer or Netscape. *Pharmabase* is housed on the Marine Biological Laboratory (MBL) server and is available entirely through Internet access. There are no specific requirements for browsers except that the browser should be relatively recent so that it can properly display PNG-formatted graphics. JavaScript is employed for some pull-down text functions, but non-JavaScript-aware browsers will display the text. Currently, the database does not employ any Flash capabilities, but in the future a Flash-enabled component will require the installation of a Macromedia Flash plug-in.

Navigating the home page

1. Open *Pharmabase* (*http://www.Pharmabase.org*). The Home Page is displayed showing the basic organizational units of the database (Fig. 14.2.1).

   At the top is a header bar, providing a link to the host site, BioCurrents Research Center, a national resource of the National Institutes of Health (NIH)/National Center for Research Resources (NCRR). Below this, on the left-hand side of the Home Page, is a frame for carrying out the first search protocol, providing access to the navigator and search

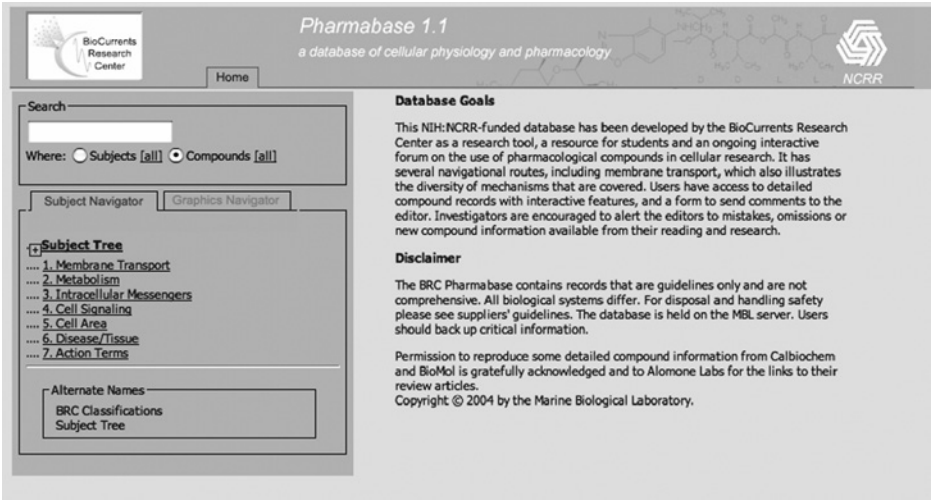
Pharmabase 1.1
a database of cellular physiology and pharmacology
BioCurrents Research Center
NCRR
Home
Search
Where: Subjects [all] Compounds [all]
Subject Navigator
Graphics Navigator
Subject Tree
1. Membrane Transport
2. Metabolism
3. Intracellular Messengers
4. Cell Signaling
5. Cell Area
6. Disease/Tissue
7. Action Terms
Alternate Names
BRC Classifications
Subject Tree
Database Goals
This NIH:NCRR-funded database has been developed by the BioCurrents Research Center as a research tool, a resource for students and an ongoing interactive forum on the use of pharmacological compounds in cellular research. It has several navigational routes, including membrane transport, which also illustrates the diversity of mechanisms that are covered. Users have access to detailed compound records with interactive features, and a form to send comments to the editor. Investigators are encouraged to alert the editors to mistakes, omissions or new compound information available from their reading and research.
Disclaimer
The BRC Pharmabase contains records that are guidelines only and are not comprehensive. All biological systems differ. For disposal and handling safety please see suppliers' guidelines. The database is held on the MBL server. Users should back up critical information.
Permission to reproduce some detailed compound information from Calbiochem and BioMol is gratefully acknowledged and to Alomone Labs for the links to their review articles.
Copyright © 2004 by the Marine Biological Laboratory.

Figure 14.2.1 *Pharmabase* Home Page illustrating the basic layout of the site and the major navigation routes. Details and disclaimers are presented as text on the right.

tools. The text on the right-hand side of the home page provides some background on Pharmabase and a disclaimer concerning its use and limitations.

Searching by compound or subject

2. To perform a direct search by compound or subject, click on the respective radio button below the Search box. For example, to search for the Vacuolar-Type Proton ATPase, select the Subject radio button and type in `Vacuolar-Type`, then hit the Enter key.

   If no result is returned, try a synonym. The following synonyms are included in Pharmabase for this proton pump: Vacuolar-Type; V-ATPase; V-Type; Vacuolar; Vacuolar Type Proton ATPase. This feature is not case-sensitive. However, omit the hyphen and the search will return zero results. After each search, the Where radio button on the Home Page defaults to Compounds. The compound search also has latitude for wording that does not quite match the database entry.

3. Select Compound radio button and type in `Bafilomycin`, then hit the Enter key. *Pharmabase* will be reduced to one compound, with the correct name "Bafilomycin A1," an antibiotic that selectively blocks the V-Type ATPase. Click on the [more] link and the Compound Record for Bafilomycin A1 is displayed. A detailed description of the Compound Record and its interpretation is given in Basic Protocol 2.

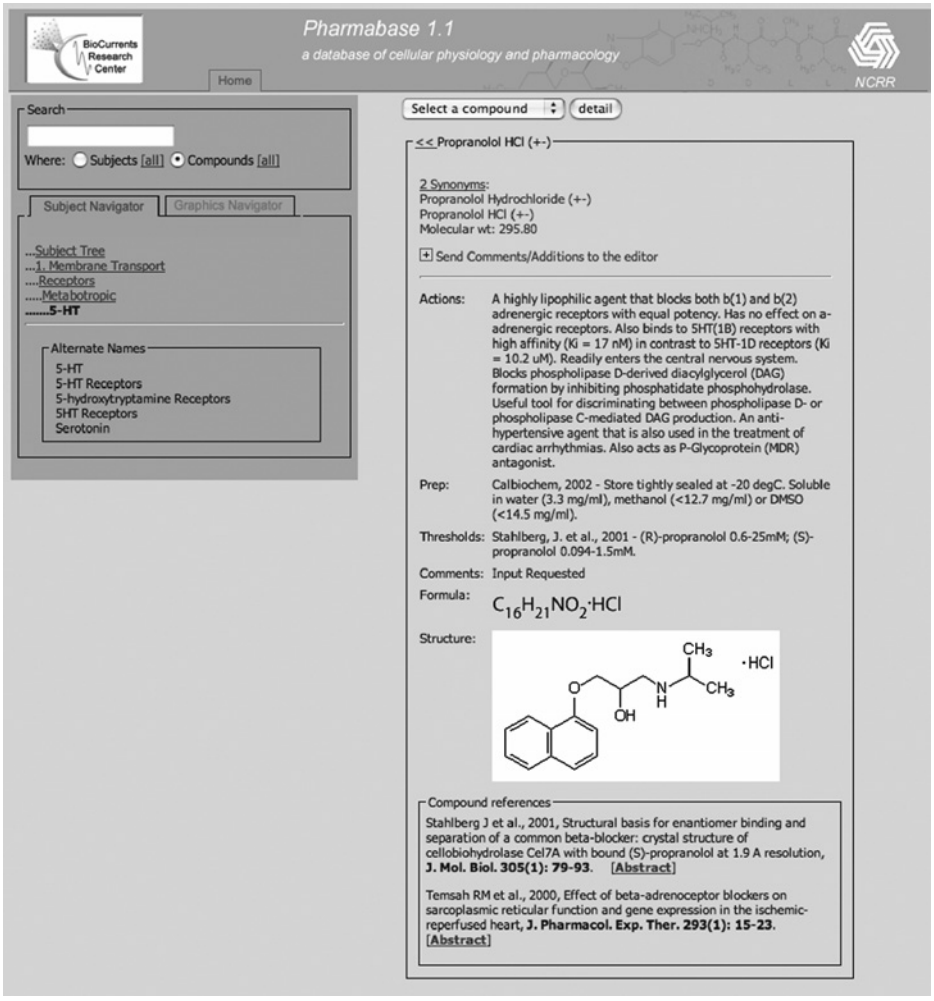
BioCurrents Research Center
Pharmabase 1.1
a database of cellular physiology and pharmacology
NCRR
Home
Search
Where: Subjects [all] Compounds [all]
Subject Navigator
Graphics Navigator
...Subject Tree
...1. Membrane Transport
....Receptors
.....Metabotropic
.....5-HT
Alternate Names
5-HT
5-HT Receptors
5-hydroxytryptamine Receptors
5HT Receptors
Serotonin
Select a compound
detail
<< Propranolol HCl (+-)
2 Synonyms:
Propranolol Hydrochloride (+-)
Propranolol HCl (+-)
Molecular wt: 295.80
Send Comments/Additions to the editor
Actions: A highly lipophilic agent that blocks both b(1) and b(2) adrenergic receptors with equal potency. Has no effect on a-adrenergic receptors. Also binds to 5HT(1B) receptors with high affinity (Ki = 17 nM) in contrast to 5HT-1D receptors (Ki = 10.2 uM). Readily enters the central nervous system. Blocks phospholipase D-derived diacylglycerol (DAG) formation by inhibiting phosphatidate phosphohydrolase. Useful tool for discriminating between phospholipase D- or phospholipase C-mediated DAG production. An anti-hypertensive agent that is also used in the treatment of cardiac arrhythmias. Also acts as P-Glycoprotein (MDR) antagonist.
Prep: Calbiochem, 2002 - Store tightly sealed at -20 degC. Soluble in water (3.3 mg/ml), methanol (<12.7 mg/ml) or DMSO (<14.5 mg/ml).
Thresholds: Stahlberg, J. et al., 2001 - (R)-propranolol 0.6-25mM; (S)-propranolol 0.094-1.5mM.
Comments: Input Requested
Formula: $C_{16}H_{21}NO_2 \cdot HCl$
Structure:
Compound references
Stahlberg J et al., 2001, Structural basis for enantiomer binding and separation of a common beta-blocker: crystal structure of cellobiohydrolase Cel7A with bound (S)-propranolol at 1.9 A resolution, J. Mol. Biol. 305(1): 79-93. [Abstract]
Temsah RM et al., 2000, Effect of beta-adrenoceptor blockers on sarcoplasmic reticular function and gene expression in the ischemic-reperfused heart, J. Pharmacol. Exp. Ther. 293(1): 15-23. [Abstract]

Figure 14.2.2 Clicking the [more] link next to a compound name opens up the Compound Record directly. Interpreting the Compound Record is discussed in Basic Protocol 2.

4. To view the entire compound database content, click the "all" link next to the Compound radio button.

   All compounds are displayed in alphabetical order on the right side. Clicking on the [more] link will lead to the Compound Record for that entry. Select the Home tab at the base of the header to return to the Home Page.

5. Select the Subjects radio button, then click the "all" link.

   All the subject headings (820 with synonyms) will now be listed on the left-hand side of the screen. Use the scroll bar on the right to view the list.

6. Scroll down the subject list and, under Receptors, subcategory Metabotropic, click the link for 5-HT.

   The database is sorted to display the navigator route to a metabotropic, membrane borne, 5-hydroxytryptamine receptor (5-HT), with alternate names on the left-hand side, and related pharmacological compounds (18 in this case) on the right-hand side. Each compound name in the list has an associated [more] link.

7. In the list of compounds on the right-hand side of the page, click the [more] link next to Propranolol HCl. The associated Compound Record for this entry is displayed, as shown in Figure 14.2.2. A detailed description on using the Compound Record is given in Basic Protocol 2.

BASIC PROTOCOL 2

USING THE SUBJECT NAVIGATOR: MEMBRANE TRANSPORT

Navigating is the key to accessing the database when the subject or compound is not known. The Navigator is presented on the left-hand side of the Home Page, below the header bar (see Fig. 14.2.1). It is divided into two sections—navigating by subject or by graphics. These points of access are the subjects of this and the following basic protocols. In summary, the point of entry into the database is selected using the navigation route. The most comprehensive is Membrane Transport, with the other subject navigators allowing a degree of preselection, and therefore reduction, of the database to subsets. The inclusion of the subset organization also allows cross-referencing between the cellular component, protein, or structure, with pharmacological tools and diseases. The Graphics Navigator (Basic Protocol 4) provides an active graphic map based on Cell Type and Pathways.

A top-down search begins with the root-level subject element or Navigator. This lists all the major subject subdivisions. Each of these subdivisions represents different taxonomies linked under a common root. Membrane Transport, the first subject category in the Subject Navigator window is the major point of entry into the database. This option deals with protein molecules responsible for the movement of ions and molecules across the lipid membranes of cells, including the plasma membrane and the membranes of organelles. Included in the context of *Pharmabase* are pumps (ATPases), channels, and porters. The latter can be symporters, uniporters, or antiporters. Also included in this category are cell membrane receptors and intercellular junctions.

Necessary Resources

Hardware

Computer with Internet access

Software

Web browser, e.g., MS Internet Explorer or Netscape. *Pharmabase* is housed on the Marine Biological Laboratory (MBL) server and is available entirely through Internet access. There are no specific requirements for browsers except that the browser should be relatively recent so that it can properly display PNG-formatted graphics. JavaScript is employed for some pull-down text functions, but

non-JavaScript-aware browsers will display the text. Currently, the database does not employ any Flash capabilities, but in the future a Flash-enabled component will require the installation of a Macromedia Flash plug-in.

Selecting "subject navigation"

1. Go to the Home Page at *http://www.Pharmabase.org*. Select the first option (no. 1 under Subject Tree), Membrane Transport. The database is queried to retrieve a list of all compounds related to this subject or any of its child nodes. The resultant number of relevant compounds is reduced from the original 717 to 453 (these numbers will change as compounds are added). Subject navigation through the Membrane Transport route is the most developed and comprehensive search route available in *Pharmabase*.

Querying the database

After the selection of Membrane Transport, two broad choices arise. The database can be queried further by making a series of choices (six in total; see below) relating to the type of transporter being considered, or the entire membrane transport structure can be exploded.

Using the "explode" function

Using the "explode" function to locate a Compound Record is primarily targeted to the investigator who knows the transporter being studied. However, there is another use for the novice, or experienced investigator looking into a new field—i.e., the explode function shows all the possible options available in *Pharmabase*, educating the user about the diversity of the database and drawing attention to categories of proteins the investigator may be unaware of.

2. Click on the plus sign ([+]) on the second line of the Navigator Window, next to "1. Membrane Transport." 133 proteins are now listed in the subject tree on the left-hand side of the window along with complete navigational subject routes. Now, for example, select Channels. Figure 14.2.3 shows the first section of the exploded Membrane Transport: Channel. All levels are searchable, but the deeper down one penetrates into the hierarchy, the more specific the query to the database. The right-hand side shows a list of compounds that relate to this level and its descendents in alphabetical order (currently 453 for membrane transport and 222 for channels).

3. Select Cations. 209 Compound Records appear on the right-hand side. Select Potassium, and the Compound Records are reduced to 85, with the Navigator presenting three subsets of potassium channels—Two-pore, Voltage-gated, and Non-Voltage-gated. The variety of potassium channels held in the database can be viewed below Potassium. 20 final potassium channel–related protein structures, associated with the 85 Compound Records, are now presented on the left-hand side with their navigational routes.

Using a "subject navigator"

Navigating the subject tree addresses the following conceptual problem: "I want to know the protein, and its pharmacology, relating to a proton gradient (pH is changing) that requires energy (ATP-dependent) but does not respond like a normal phosphorylating pump (such as the Na^+/K^+-ATPase superfamily), as our target is vanadate-insensitive. How do I reduce my options?" On the left-hand side of the page, under Subject Tree, is the route taken through the Subject Navigator. Below this, synonyms (alternate names) are listed along with links, where available, to gene banks and Web-based structural information. This section is incomplete, subject to continuing enhancements. In most cases, these links will lead to the NCBI Entrez Gene bioinformatics project.

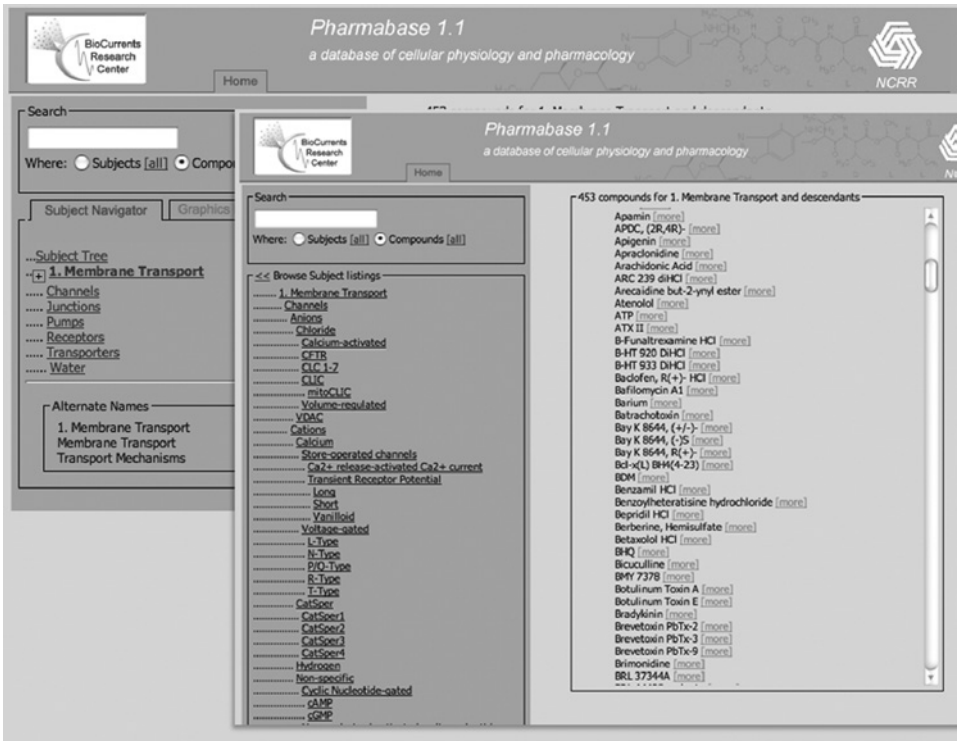

Figure 14.2.3 An example of the expanded subject listing after exploding the Subject Tree. Membrane Transport was selected from the Subject Navigator on the Home Page.

This resource of the National Library of Medicine (NIH) provides material on the related gene sequences for the molecule in question. Additionally, it provides numerous links to other sites related to the chosen target. Links to the Kyoto Encyclopedia of Genes and Genomes (KEGG; *UNIT 1.12*; *http://www.genome.ad.jp/kegg/pathway.html*) are particularly relevant to *Pharmabase*. Two other sites contain some of this information, providing additional links. These are *http://www.rcsb.org/pdb/* (*UNIT 1.9*) and *http://www.tcdb.org*. Also see the Worldwide Protein Data Bank at *http://www.wwpdp.org/index.html*.

4. At the Home Page, select Subject Navigator from the two tabs above the Subject Tree.
5. Select Membrane Transport.
6. From the subset list select Pumps. Pumps are defined as transporters utilizing the energy stored in the phosphate bond of ATP; as a group they are referred to as the ATPases. An alternative name for the term Pumps, "ATPase," is displayed below the classification. Making this selection reduces the list to 10 structures and 34 compounds. The structure list can be viewed by selecting the explode option ([+]) next to Pumps in the Subject Navigator. All 34 compounds are listed on the right and can be viewed using the scroll bar.
7. Select Non-phosphorylating. In eukaryotes, Hydrogen is the only product of this selection.
8. Select Hydrogen to reveal two variants of a non-phosphorylating hydrogen (proton) pump. As the F1F0 is mitochondrial-based and does not regulate acidification (it is the ATP synthase), choose the Vacuolar-Type. The navigation route described is shown numbered in Figure 14.2.4.

9. Clicking on Vacuolar-Type displays the view shown in Figure 14.2.5, and the three compounds that have Compound Records associated with this protein are shown on the right-hand side: Bafilomycin A1, Concanamycin A, and N-Ethylmaleimide (NEM).

   The first two compounds, antibiotics, are very specific for this pump, whereas NEM has problems. Although one would no longer use NEM to investigate V-Type activity, for some time it was the only available blocker. The Compound Record remains useful for demonstrating the Pharmabase content.

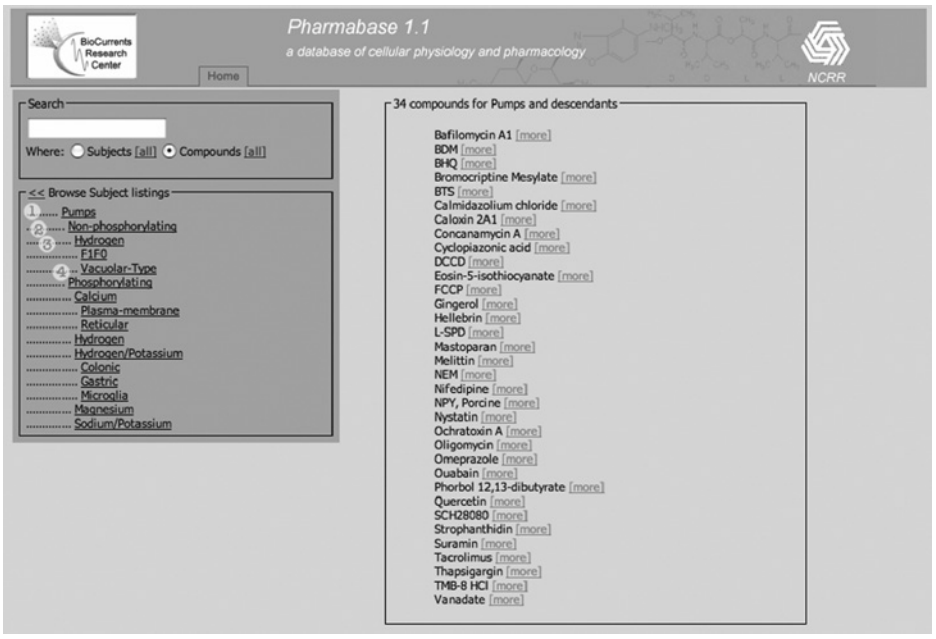

Figure 14.2.4 Example of using the Subject Navigator to descend through the hierarchical tree via a series of simple choices.

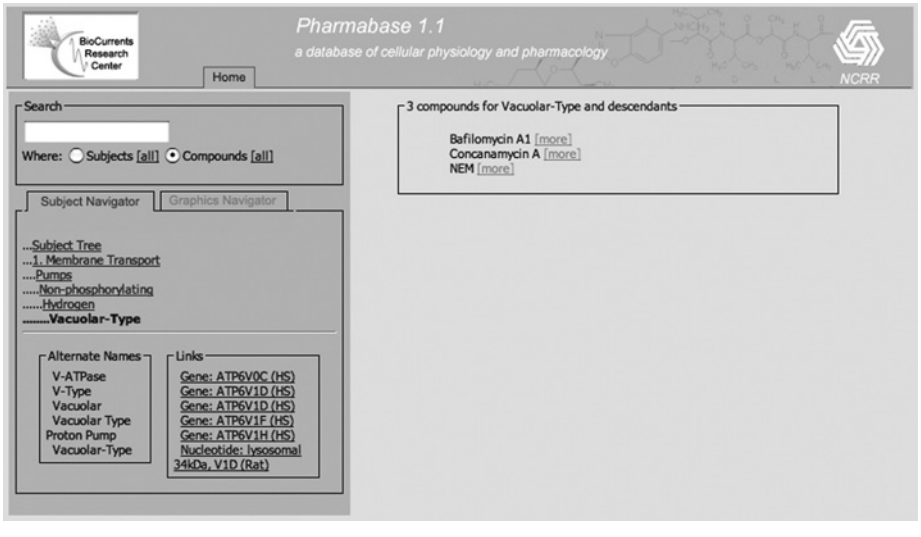

Figure 14.2.5 The Web page presented when the end of the hierarchical tree for the Vacuolar-Type Proton Pump is reached. To the left is the route, to the right, three compounds that target the V-Type.

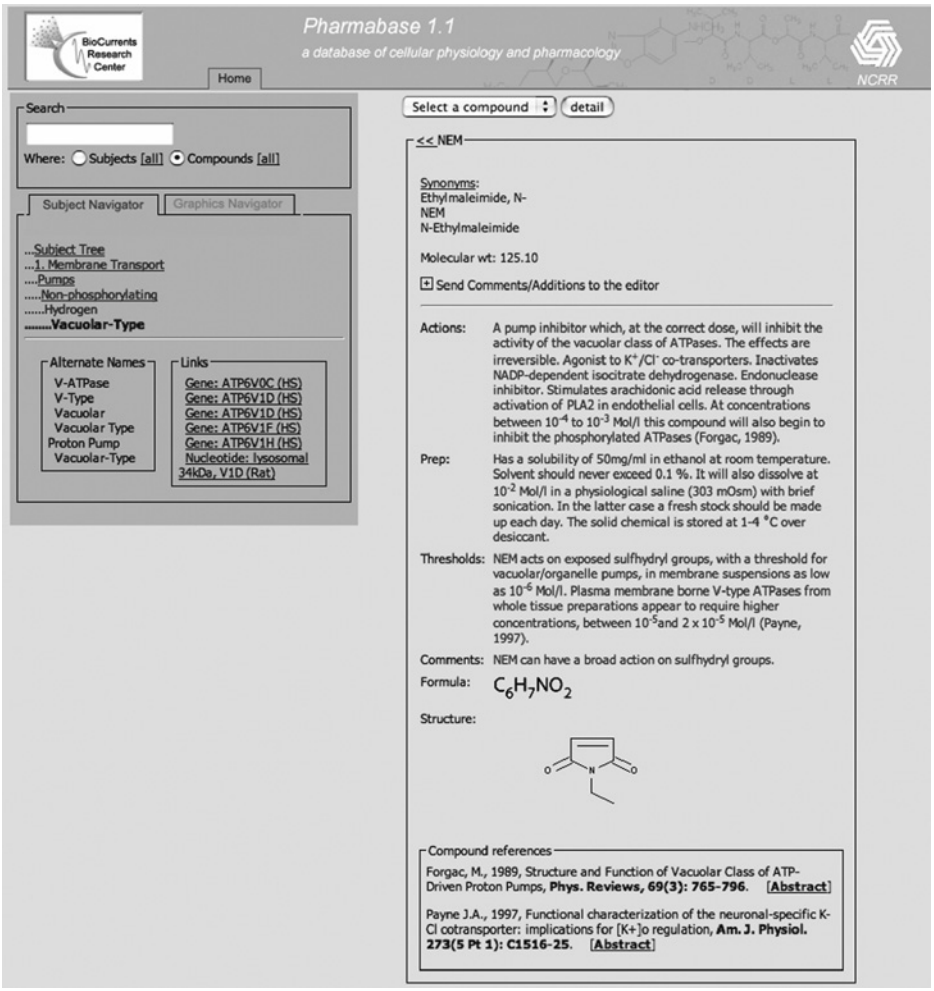
Pharmabase 1.1
a database of cellular physiology and pharmacology
BioCurrents Research Center
NCRR
Home
Search
Where: Subjects [all] Compounds [all]
Subject Navigator
Graphics Navigator
...Subject Tree
...1. Membrane Transport
....Pumps
.....Non-phosphorylating
.....Hydrogen
.....Vacuolar-Type
Alternate Names
V-ATPase
V-Type
Vacuolar
Vacuolar Type
Proton Pump
Vacuolar-Type
Links
Gene: ATP6V0C (HS)
Gene: ATP6V1D (HS)
Gene: ATP6V1D (HS)
Gene: ATP6V1F (HS)
Gene: ATP6V1H (HS)
Nucleotide: lysosomal 34kDa, V1D (Rat)
Select a compound
detail
<< NEM
Synonyms:
Ethylmaleimide, N-
NEM
N-Ethylmaleimide
Molecular wt: 125.10
Send Comments/Additions to the editor
Actions: A pump inhibitor which, at the correct dose, will inhibit the activity of the vacuolar class of ATPases. The effects are irreversible. Agonist to K+/Cl- co-transporters. Inactivates NADP-dependent isocitrate dehydrogenase. Endonuclease inhibitor. Stimulates arachidonic acid release through activation of PLA2 in endothelial cells. At concentrations between 10-4 to 10-3 Mol/l this compound will also begin to inhibit the phosphorylated ATPases (Forgac, 1989).
Prep: Has a solubility of 50mg/ml in ethanol at room temperature. Solvent should never exceed 0.1 %. It will also dissolve at 10-2 Mol/l in a physiological saline (303 mOsm) with brief sonication. In the latter case a fresh stock should be made up each day. The solid chemical is stored at 1-4 °C over desiccant.
Thresholds: NEM acts on exposed sulfhydryl groups, with a threshold for vacuolar/organelle pumps, in membrane suspensions as low as 10-6 Mol/l. Plasma membrane borne V-type ATPases from whole tissue preparations appear to require higher concentrations, between 10-5and 2 x 10-5 Mol/l (Payne, 1997).
Comments: NEM can have a broad action on sulfhydryl groups.
Formula: C6H7NO2
Structure:
Compound references
Forgac, M., 1989, Structure and Function of Vacuolar Class of ATP-Driven Proton Pumps, Phys. Reviews, 69(3): 765-796. [Abstract]
Payne J.A., 1997, Functional characterization of the neuronal-specific K-Cl cotransporter: implications for [K+]o regulation, Am. J. Physiol. 273(5 Pt 1): C1516-25. [Abstract]

Figure 14.2.6 The Compound Record for N-ethylmaleimide (NEM), one of the agents listed in Figure 14.2.4 as targeting the V-Type. See the text for a description of the Record structure.

10. Click on the [more] option next to NEM to access the Compound Record for that entry. The current page is replaced to include the Compound Record associated with NEM and the Vacuolar-Type (Fig. 14.2.6).

Interpreting the compound record

11. The Compound Record shown in Figure 14.2.6 is divided into four sections. The header bar and the compound or subject search area to the left are described in Basic Protocol 1. Below the search area, the Navigator area containing the navigation route is found. Below this are synonyms, if appropriate, and links to gene and structural information. Plans have been made to include a graphics window below the synonyms. This window will provide structural information and related site links with graphic content. On the right-hand side is information concerning the compound—in this case, *N*-ethylmaleimide.

12. The Compound Record presents the main information contained in *Pharmabase*. Three sections are incorporated.

    a. A header with definitions—compound name, synonyms, and molecular weight. This also includes a simple way to contact the *Pharmabase* Editor.

        This feature is to encourage input from the user. As the database tries to remain current and continually develops, input from the user group is invaluable. Some records are incomplete or do not recognize a nonselectivity known to others. More relevant references may be available.

    b. An information block, including the compound formula and structure.

    c. Specimen references.

        Where applicable the scroll bar to the right of the page allows full access to the information and bibliography.

13. The information block contains details on the compound of particular use to the experimentalist. It is divided into the following sections.

    a. *Action:* This section defines the targets of the compound and its actions—for example, inhibitory (antagonistic) or agonistic. For NEM there are a number of possible actions; obviously, if the investigator is unaware of these, mistakes can be made. Notable is the action on the V-Type at micromolar concentrations, but there is also an action on phosphorylating ATPases at millimolar levels. Furthermore, as the compound generally attacks sulfhydryl groups, it can impact a broad number of mechanisms, inevitably more than listed.

    b. *Preparation:* Where the Merck Index gives solubility from a chemist's point of view, *Pharmabase* puts this in a biological context. For biological investigations, concentrations at or above the biological threshold are of interest, rather than maximum solubility. For example, NEM has a very poor water solubility, but can be prepared with sonication at levels needed to inhibit the V-Type. This avoids problems with solvent toxicity. This section points out that nonaqueous solvent concentrations should not exceed 0.1%.

    c. *Thresholds:* Where available, this gives the final concentrations needed to block the action of a target protein.

    d. *Comment:* This provides a space for drawing attention to features not dealt with above or which need to be emphasized—in this case, the clear lack of specificity of NEM, which manifests a general action on sulfhydryl groups. This lack of specificity, particularly with regard to applied concentrations and thresholds, cannot be overemphasized in the use of any pharmacological compound. This will be further discussed in the Commentary. In some of the data records there is also a Problems section, which is being absorbed into the Comment and Action fields.

        Where no compounds are associated with a target molecule, there may be more generic compounds for a transporter or channel group listed at a higher level.

SEARCHING *PHARMABASE* BY BIOCHEMICAL PATHWAY OR CELL STRUCTURE TARGETS

BASIC PROTOCOL 3

In this protocol, four other subject-based Navigators are described. These are organized into the following categories: Metabolism, Intracellular Messengers, Cell Signaling, and Cell Area.

Necessary Resources

Hardware

Computer with Internet access

Software

Web browser, e.g., MS Internet Explorer or Netscape. *Pharmabase* is housed on the Marine Biological Laboratory (MBL) server and is available entirely through Internet access. There are no specific requirements for browsers except that the browser should be relatively recent so that it can properly display PNG-formatted graphics. JavaScript is employed for some pull-down text functions, but non-JavaScript-aware browsers will display the text. Currently, the database does not employ any Flash capabilities, but in the future a Flash-enabled component will require the installation of a Macromedia Flash plug-in.

1. On the Home Page (*http://www.Pharmabase.org*), the Subject Navigator provides a choice of seven navigational routes listed under Subject Tree. In Basic Protocol 2, navigational route number 1, Membrane Transport, was addressed. This protocol collectively addresses numbers 2 to 7. All of these categories are in the early stages of development, with editing and expansion planned.

   These routes provide access to subsets of Pharmabase, reducing the Compound Records to targeted areas.

2. *Metabolism (no. 2).* This category will focus on the steps behind the cellular processing of metabolites, such as glucose and the production of ATP. For more detail, see steps 7 to 12.

3. *Intracellular Messengers (no. 3) and Cell Signaling (no. 4).* These should currently be considered together, encompassing the mechanisms by which information is conveyed across the cytosolic component of a cell. These mechanisms can couple membrane receptors to cellular action and/or gene expression. For more detail, see steps 13 to 15.

4. *Cell Area (no. 5).* This field is self-explanatory allowing a user to select a cell region, structure, or organelle.

5. *Diseases and Tissues (no. 6).* These categories will allow the database to be presorted to molecules and compounds specific to certain disease states and tissues with specialized expression patterns.

6. *Action Terms (no. 7).* This category address compounds by their action on the target, for example, whether they are agonists or antagonists, solvents, permeabilizers, or reporter molecules.

Searching metabolism

The following steps are carried out from the Home Page (*http://www.Pharmabase.org*) in the Subject Navigator window.

7. Select Metabolism. 53 compounds are selected from the database. These are associated with a current index under metabolism in the Navigator Window of 7 processes.

8. Select ATP. 13 compounds are associated at this level with only one further option presented.

9. Select Production. 6 compounds address this level, each with its own Compound Record.

10. Click on [more] next to FCCP, and the Compound Record is displayed on the right-hand side. The format is the same as discussed above for NEM (Basic Protocol 2). As in many cases, this compound has multiple actions depending on the target and concentration. Most commonly, it is used as a protonophore to depolarize the mitochondrial membrane by creating a proton leak. This dissipates the proton gradient

used to drive the ATP synthase termed the F1F0 pump. This information is contained in the first text field, Action, of the Compound Record. However, having come in via a subset route, the investigator may not know what the F1F0 is. This can be resolved by using the Basic Protocol 1 component search by subject.

11. In the Search Window above the Navigator (also see Basic Protocol 1) select the Subject radio button and type in `F1F0`, the press the Enter key. The page refreshes to present one match below the Search Window—F1F0.

12. Select "F1F0." The window refreshes to the Subject Navigator and the route through the Membrane Transport protocol discussed in Basic Protocol 2. Below the Navigator Route are displayed alternate names and links to gene sequence, where listed and available. To the left, the selection of Compound Records associated with the transporter is displayed.

    Four Compound Records are displayed in this case, one being FCCP.

Searching intracellular messengers and cell signaling

Navigational Routes 3 and 4 of *Pharmabase* (see above) should be handled as one. There is currently considerable thematic overlap between the categories Intracellular Messenger and Cell Signaling. Future development will refine these. For example, from the Subject Tree area of the Home Page:

13. Select Intracellular Messengers. This action presents three further choices:

    a. Intermediates
    b. Messengers
    c. Receptors.

14. Select Intermediates. A limited selection of 11 kinase molecules are presented. There are 105 compounds associated. Return to the Subject Tree on the Home Page.

15. Select Cell Signaling. This choice reveals six subjects related to 38 compounds. Selecting Phospholipase A2 reduces the compound list to 20.

    Cell signaling, particularly when referring to signal transduction mechanisms, is complex. As yet, Pharmabase does not include an adequate set of navigational tools or specific Compound Records. For interested parties, other databases allow entry into this field. Examples are:

    The Database of Quantitative Cellular Signaling (http://doqcs.ncbs.res.in/). This is a repository of models of signaling pathways. Included are reaction schemes, concentrations, and rate constants, as well as annotations on the models.

    Another site, available through subscription, is the Signal Transduction Knowledge Environment (stke; http://stke.sciencemag.org/) run by the American Association for the Advancement of Science.

    The Protein Kinase Resource (PKR; http://pkr.sdsc.edu/html/index.shtml) aims to be a Web compendium of information on the protein kinase family of enzymes. The PKR is a collaborative project of researchers and computational biologists working to integrate molecular and cellular information.

    Cell Signaling Technology (http://www.cellsignal.com), a company site, provides a searchable set of "kinomes" where, like the Pharmabase Graphics Navigator (see Basic Protocol 4), the pathway components are clickable allowing access to the company's product catalog.

Search cell area

The final subset of Navigational Routes is Cell Area, category 5 in the Subject Tree. Here, compounds noted to target particular cellular components are associated at that level. For example, from the Subject Tree on the Home Page *http://www.pharmabase.org*.

16. Select Cell Area. A list of 13 subcellular structures are presented within the Navigator. 212 compounds are associated at this level.

17. Select Reticulum. Two choices are now available, the endoplasmic and the sarcoplasmic reticulum.

18. Select Endoplasmic Reticulum. A list of 21 compounds is presented on the right-hand side. Click on [more] next to Caffeine and the Compound Record now occupies the right-hand side. The protein targets for Caffeine are listed in the Actions of the Compound Record.

    As with a search through Metabolism (see above), these target molecules may be unfamiliar to an investigator entering the database through a subset navigation route. Additional information may thus be obtained, e.g., as in step 19, using the example of the ryanodine receptor mentioned as a target under Actions in the Compound Record for Caffeine.

19. Select the Subjects radio button in the Search area. Type in `ryanodine receptor` and hit the Enter key. One entry is found (indicated by a link underneath the Search area). Clicking on this link presents three receptor types, and the navigation route via subset 3, Intracellular Messengers. 12 compounds, with access to the Compound Records via the [more] link, are associated at this level. Selecting the Type (via the links under the Subject Tree area of the window) can generate compounds selective for that molecule.

Other searches

As subsets 6 and 7 are very much in their infancy, no details to their use are given here.

20. Subset 6 in the Subject Navigator aims to address compounds relevant to diseases or tissues. This hierarchy currently extends to only one level. A more complete hierarchy of diseases and physiological conditions related to tissues will become available as the database grows.

    An example from this subset is Apoptosis to which 63 compounds are mapped as related to this choice.

21. Subset 7 allows compounds to be searched according to their Action on the target.

BASIC PROTOCOL 4

USING THE GRAPHIC NAVIGATOR: SEARCHING CELL TYPE OR PATHWAY

In addition to the hierarchically orientated navigation by subject described in the basic protocols above, *Pharmabase* offers a graphics interface. The Graphics Navigator is a relational search method. Proteins are arranged in pathways such that their interrelationship is apparent. This Navigator is also a work in progress, with Figure 14.2.7 illustrating and example of the appearance of the searchable window. The Graphics Navigator is organized with a left-hand searchable panel starting with either Cell Type or Pathway. In the example, selecting Cell Type is further reduced, e.g., as follows:

1 Cell Type
1.1 Beta Cell (pancreas)
1.1.a ATP production and membrane depolarization
1. 1.a.1 F1F0.

The Graphics Navigator is anticipated to be a powerful search tool. Currently, the model under construction is for the pancreatic beta cell and glucose-stimulated insulin release.

Necessary Resources

Hardware

Computer with Internet access

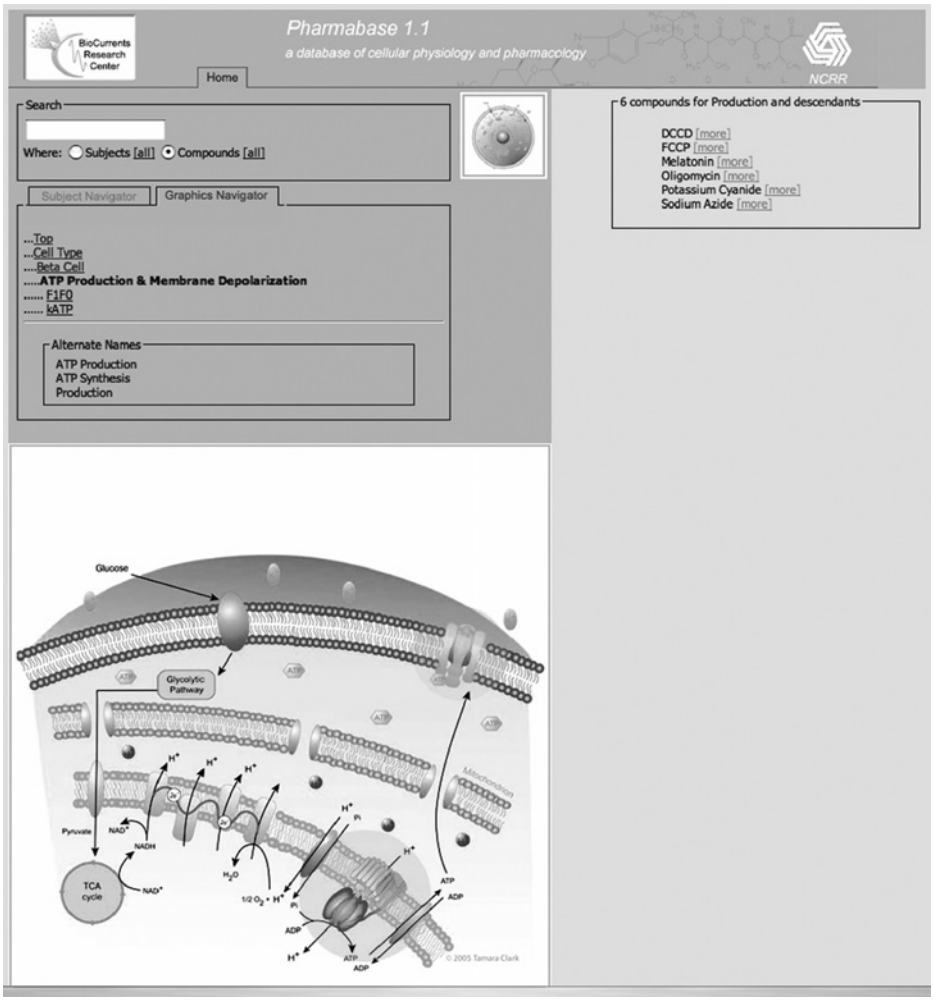

Figure 14.2.7 The Graphics Navigator illustrating the first searchable pathway of the pancreatic beta cell. Clicking the F1F0 pump on the mitochondrial membrane moves on to Figure 14.2.8. For the color version of this figure go to *http://www.currentprotocols.com*.

Software

Web browser, e.g., MS Internet Explorer or Netscape. *Pharmabase* is housed on the Marine Biological Laboratory (MBL) server and is available entirely through Internet access. There are no specific requirements for browsers except that the browser should be relatively recent so that it can properly display PNG-formatted graphics. JavaScript is employed for some pull-down text functions, but non-JavaScript-aware browsers will display the text. Currently, the database does not employ any Flash capabilities, but in the future a Flash-enabled component will require the installation of a Macromedia Flash plug-in.

Using the "graphics navigator"

1. Select Graphics Navigator on the Home Page (*http://www.Pharmabase.org*). From the links in the window that appears, select Cell Type.
2. Select Beta Cell (the only choice at time of writing).

3. Pass the cursor over the cell graphic; five active pathways are embedded to date. Select the first pathway by clicking on the mitochondrion or its surrounding area.

   This action will bring up the Pathway Graphic below the navigation route and synonyms. Immediately to the right of the Search window is a thumbnail of the "Beta Cell" (Fig. 14.2.7). Clicking on this or the Beta Cell text returns the user to the higher level of the Graphics Navigator.

4. Pass the cursor over the Pathway Graphic. Two components are currently linked to the database: i.e., the F1F0 pump and the ATP-dependent potassium channel. Active links are currently indicated by the red highlighting circle. Leave the cursor over one of the linked images and a descriptor pop-up appears.

5. Find the F1F0 and select by clicking on it.

   The Pathway Graphic now settles as a thumbnail beneath the Cell Graphic, and is replaced by a graphic of the targeted molecule. Below the Navigator are synonyms and links to gene structure. The database is sorted to compounds targeting the F1F0 with these being presented on the right-hand side.

6. Click the [more] link next to FCCP. The compound listing is replaced by the Compound Record for FCCP (Fig. 14.2.8). For a guide through the Compound Record, refer to Basic Protocol 2.

Pharmabase 1.1
a database of cellular physiology and pharmacology
BioCurrents Research Center
NCRR
Home
Search
Where: Subjects [all] Compounds [all]
Subject Navigator
Graphics Navigator
...Top
...Cell Type
....Beta Cell
.....ATP Production & Membrane Depolarization
.....F1F0
Alternate Names
F1F0
Links
Gene: ATP5H (HS)
Gene: Atp5k (Mus)
Gene: ATP5L (HS)
Gene: RGD:621377 (Rat)
Nucleotide: ATP5O (HS)
Select a compound
detail
<< FCCP
Synonyms:
FCCP
Carbonyl cyanide p-(trifluoromethoxy)phenylhydrazone
Molecular wt: 254.20
Send Comments/Additions to the editor
Actions:
FCCP is a protonophore (H+ ionophore) in both mitochodria and the plasma membrane. It will depolarize the mitochondrial membrane, releasing calcium and preventing uptake, and uncoupling oxidative phosphorylation. Its action will increase cellular oxygen consumption but will reduce production of ATP through indirect inhibition of the F1F0 pump as a result of collapsing the mitochondrial hydrogen ion gradient. It also is reported to inhibit a background K+ current and induce a small inward current, reduce pH by 0.1 unit, and induce a rise of intracellular [Na+]. FCCP stimulates Mg2+-ATPase activity, inhibits β-amyloid production, and mimics the effect of selective glutamate agonist N-methyl-D-aspartate (NMDA) on mitochondrial superoxide production.
Prep:
Soluble in DMSO (Biomol, 2001) or 95% Ethanol (Sigma-Aldrich, 2005). Solvent should never exceed 0.1%. Store, as supplied, at room temperature for up to 1 year. Store solutions at -20°C for up to 3 months (Biomol, 2001). This compound will adhere to plastic and glass and experimental chambers should be thoroughly washed in alcohol and/or soaked in BSA.
Thresholds:
Action on mitochondria - <=0.025-1 uM; action on plasma membrane - 1 μM (Tretter, L. et al, 1998). Treatment of FCCP at varying concentrations leads to partial (100 nM) or complete (10 μM) depolarization and apoptosis.
Comments:
Formula:
C10H5F3N4O
Compound references
Tretter L et al., 1998, Plasma membrane depolarization and disturbed Na+ homeostasis induced by the protonophore carbonyl cyanide-p-trifluoromethoxyphenyl-hydrazon in isolated nerve terminals, Mol. Pharmacol. 53(4): 734-41. [Abstract]

Figure 14.2.8 The terminal point for the transporter F1F0 and targeted compounds after selection of the compound FCCP. The Compound Record is displayed to the right. For the color version of this figure go to *http://www.currentprotocols.com*.

COMMENTARY

Background Information

Pharmabase provides an educational and research tool accessible to a broad spectrum of investigators and students. Navigating the database can be approached from different levels and assumes varying degrees of background knowledge. In all cases, the final result is a compound data sheet. The user can, however, utilize the linking capacity at various steps to jump to other databases related to the level of search. The broad goal is to direct the user to the correct selection and use of pharmacological compounds targeting cellular proteins and their function in living cells.

It is worthwhile to consider what the major obstacles are to the successful use of a pharmacological compound and how a bioinformatics approach, such as *Pharmabase*, helps to solve them.

1. First, and foremost, the researcher needs to identify a target, sometimes with minimal information. Here, a directed knowledge base, indicating what molecules exist for moving an ion or compound and what varieties are present in a particular tissue or within a known pathway, can be of advantage to the experienced investigator. *Pharmabase* tackles this problem by presenting two alternative search mechanisms—one subject-based and the other graphics-based. The subject-based approach allows several routes of access to the database, such as through transporters, diseases, or structures. A major advantage here is that the whole subject index can be viewed and related to function. This reveals the diversity of mechanisms available without any prior knowledge being required. In the graphics approach, a relational database is presented. Pathways or cell types are accessed where different molecules, embedded in the same pathway, can be accessed. No prior knowledge of the pathway is needed.

2. Once a target is selected, a compound must be chosen. The core of *Pharmabase* is the joining of the search, or navigator, routes with compounds that alter the performance of a chosen target or category of targets. Each compound, arrived at by the selective reduction of the compound listing with more targeted selections, has a compound record.

3. One critical issue with almost all pharmacological compounds is the matter of selectivity. Almost always, if used incorrectly, a compound can have multiple targets. This may be a result of similar binding sites and/or chemistry of the susceptible protein structures. Here the experienced investigator has an advantage in knowing the "tricks of the trade." A key component of the Compound Record in *Pharmabase* draws these caveats to the user's attention.

4. Once the molecule to be studied has been identified, and a suitable compound has been selected to modify its performance, it is necessary to know the concentrations at which to apply it and how it should be handled—in terms of solubility, storage, etc. The *Pharmabase* Compound Record has separate fields that deal with these issues, and also provides selected references. Of note is the disclaimer that records can be incomplete, and, importantly, that any user should consult the manufactures guidelines with regard to toxicity and safe handling. Many of the compounds listed in the database are hazardous.

Figure 14.2.9 illustrates the organizational layout of *Pharmabase*. A key element is that the Subject and Graphics Navigators address the same databases, cross-indexing between routes and subsets. Unseen to the user is the data management and editing capability. *Pharmabase* editors use this tool to add and edit compound information, link compound records to the bibliography, and manage compound synonymy. The Editor is also used to manage the subject indexes, and contains tools for linking compounds to the various taxonomies provided in *Pharmabase* and for managing the hierarchical and relational taxonomies themselves. Compounds can be linked to or unlinked from any node in a classification hierarchy.

Editors can also make structural changes to a subject term. Subjects can be inserted into an existing hierarchy or moved to a new location. Synonyms can be swapped to create a new preferred display term or the node can be removed altogether.

The flexibility of the Editor allows the relational databases and Navigators to be easily updated and corrected. This capability makes the interactive goal of *Pharmabase* feasible. Users are encouraged to supply additional information, correct inaccurate entries or omissions, and request additional navigational routes and graphics.

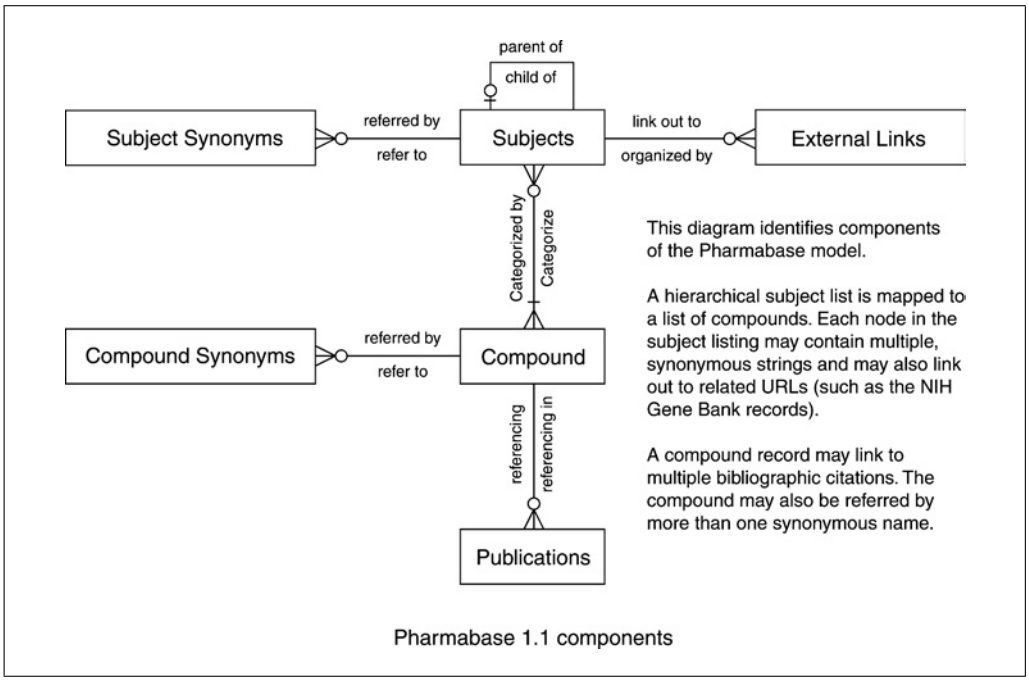
parent of
child of
Subject Synonyms
referred by
refer to
Subjects
link out to
organized by
External Links
Categorized by
Categorize
This diagram identifies components of the Pharmabase model.
A hierarchical subject list is mapped to a list of compounds. Each node in the subject listing may contain multiple, synonymous strings and may also link out to related URLs (such as the NIH Gene Bank records).
Compound Synonyms
referred by
refer to
Compound
referencing
referencing in
A compound record may link to multiple bibliographic citations. The compound may also be referred by more than one synonymous name.
Publications
Pharmabase 1.1 components

Figure 14.2.9 The structural components of *Pharmabase*.

Critical Parameters and Troubleshooting

The core information for *Pharmabase* lies in the Compound Record database. This database was started to make up for a shortfall in the published literature. Scientific research papers, under the onerous pressure of limited space, are frequently parsimonious with details on compound actions and preparations. Solubility and selectivity are often omitted; even thresholds can be missing. Investigators must either be conversant with the compound and target molecule or have access to in-depth studies, such as a recent publication on calcium channel pharmacology (McDonough, 2003). A novice to a field can, however, find advanced texts hard to digest and general texts inadequate in detail. The alternative mechanism is to trawl through the primary literature, necessitating patience and an excellent library resource. *Pharmabase* seeks to bypass these problems and cross-index the databases to each other. However, the scope of the task should not be underestimated, and the problems with available data should always be borne in mind. It is safe to expect nonspecificity from any pharmacological compound, and, as *Pharmabase* grows, more of these issues will be comprehensively covered. But beware—if an experimental result is clearly inconsistent or paradoxical, it is wise to question the selectivity before rewriting the literature!

The problem of tissue or species variability is of a similar nature to that of compound specificity. Different molecules, supposedly similar in function, can have a variable pharmacology. For example, schistosomiasis is a disease caused by trematode flatworms of the genus *Schistosoma*. A relatively common tropical disease, it can be easily and cheaply treated with the drug praziquantel (PZQ). For some time the target of this drug was unclear but Greenberg and colleagues (see Greenberg, 2005, for review) have shown that a beta subunit of a voltage-sensitive calcium channel confers PZQ sensitivity. The unique sequence of this subunit in the schistosomes imparts the selectivity of the drug. Because this is the only commercially available agent targeting these parasites, the possibility of resistance developing in the organisms, indicated by these findings, is frightening; hence, more alternatives are needed. This is a perfect example of where the molecular and pharmacological sciences can converge, with important consequences to the understanding of diseases and their threats, as well as the future ability to control such diseases. Species differences can also tell evolutionary stories. A good example is the mongoose, a predator with a particular appetite for poisonous snakes. Here, the structure of the nicotinic acetylcholine receptor has been modified to make it resistant to the alpha neurotoxins, including alpha bungarotoxin, which comes from the Krait, a poisonous snake that is a favorite meal of the mongoose (Barchan et al., 1992). Sequence information related to this example is available through the Expert Protein Analysis System (*http://au.expasy.org/uniprot/P54251*).

Acknowledgments

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Literature Cited

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Greenberg, R.M. 2005. Are Ca^{2+} channels targets of praziquantel action? *Int. J. Parasitol.* 35:1-9.

McDonough, S.I. 2003. Peptide toxin inhibition of voltage gated calcium channels: Selectivity and mechanisms. *In* Calcium Channel Pharmacology, 1st ed. (S.I. McDonough, ed.). Plenum, New York.

Rose, M.R. and Griggs, R.C. 2001. Channelopathies of the Nervous System, p. 347. Butterworth-Heinemann, Burlington, Mass.

Internet Resources

Diversity in molecular channels and transporters

http://www.tcdb.org

The Transport Classification Database provides information on the diversity of these mechanisms and links to gene sites.

Transporters and channels in disease

http://www.channelopathies.org/

Channelopathies, maintained by the University of Ulm, provides an overview of channelopathies, organized for patients, doctors, and researchers.

http://www.neuro.wustl.edu/neuromuscular/mother/chan.html#SCN4A

The Neuromuscular Disease Center provides an extensive, cross-referenced, database of ion channels, transmitters, and receptors, and their role in disease.

Protein structure databases

http://www.rcsb.org/pdb/cgi/resultBrowser.cgi

Protein Data Bank.

http://www.wwpdp.org/index.html

The Worldwide Protein Data Bank.

http://au.expasy.org/

The ExPASy (Expert Protein Analysis System) proteomics server of the Swiss Institute of Bioinformatics (SIB) is dedicated to the analysis of protein sequences and structures as well as 2-D PAGE.

Signal transduction pathways

http://doqcs.ncbs.res.in/

The Database of Quantitative Cellular Signaling: a repository of models of signaling pathways. Included are reaction schemes, concentrations, and rate constants, as well as annotations on the models.

http://stke.sciencemag.org/

The Signal Transduction Knowledge Environment (stke) run by the American Association for the Advancement of Science. This requires a subscription.

http://pkr.sdsc.edu/html/index.shtml

The Protein Kinase Resource (PKR) has as its aim to be a Web compendium of information on the protein kinase family of enzymes. The PKR is a collaborative project, of researchers and computational biologists working to integrate molecular and cellular information.

http://www.cellsignal.com.

Cell Signaling Technology provides a searchable set of "kinomes" where, like the Pharmabase "Graphics Navigator" (Basic Protocol 4), the pathway components are clickable, allowing access to the company's product catalog.

http://www.emdbiosciences.com/html/EMD/interactivepathways.htm

Calbiochem also offers a variety of interactive pathways to help find the products directed against various cell components—the emphasis is on signal transduction.

Proteomic and genomic databases

http://www.ncbi.nlm.nih.gov/gquery/gquery.fcgi

A general database focusing on several genetic and protein problems relevant to this protocol can be found at Entrez, The Life Sciences Search Engine managed by the NCBI.

Key References

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A text that covers channels, receptors, and gap junctions, as related to disease.

Hille, B. 2001. Ion Channels of Excitable Membranes. Sinauer Associates, Inc., Sunderland, Mass.

An advanced biophysical text that covers channel properties in excitable cells.

Piccolino, M. 1997. Luigi Galvani and animal electricity: Two centuries after the foundation of electrophysiology. *Trends Neurosci.* 20:443-448.

A general introduction, from an historical perspective, to excitable membranes.

Stein, W.D. 1990. Channels, Carriers, and Pumps. Academic Press, Inc., San Diego, Ca.

Although now dated, this is an excellent and well written introduction to the field of transmembrane transport mechanisms.

Contributed by Peter J. S. Smith and David Remsen
BioCurrents Research Center
Marine Biological Laboratory
Woods Hole, Massachusetts

Using MSDchem to Search the PDB Ligand Dictionary

The Protein Data Bank (PDB; *UNIT 1.9*) is an extremely valuable resource for understanding the three-dimensional (3-D) structure of proteins and interacting ligands. The PDB datafiles, however, do not provide clear and unambiguous information about chemical properties (e.g., bond orders, atom elements, and charges) for biological molecules. The possible ways that atoms in the molecules entered in the PDB are connected to form ligands and polymer residues are calculated from atom distances in the 3-D space. This is exactly what many protein visualization packages do—successfully in most, but not all, cases. Experimental errors and inaccuracies complicate things further; as a result, information about important chemical characteristics such as aromatic rings and chiral atoms is not directly accessible to scientists who want to understand the chemical structure of ligands they encounter in a PDB file.

The Macromolecular Structure Database (MSD), one of three that maintains the Worldwide Protein Data Bank (wwPDB), provides MSDchem, the definitive database of chemical records of PDB ligands (Bernstein et al., 1977). MSDchem contains data supplementary to the PDB archive that is exchanged among members of wwPDB (Berman et al., 2005; Golovin et al., 2004). These data provide explicit chemical definitions for standard and modified amino acids, nucleic acids, drugs, inhibitors, cofactors, and other chemical species included in PDB entries.

MSDchem is of use to structural biologists who want to resolve the chemical identity of a small molecule's 3-D structure and to chemists who are interested in a ligand's biological structure and function. MSDchem utilizes chemical software packages and resources including CACTVS (Ihlenfeldt et al., 1992; *http://www2.chemie.uni-erlangen.de/software/cactvs*) and CORINA (Gasteiger et al., 1990; *http://www.mol-net.de/software/corina*). The CACTVS toolkit implements several checks on chemical consistency and functions to introduce additional molecular properties such as explicit stereodescriptors, aromatic flags, chemical drawings with PDB atom names, and unique SMILEs strings (Weininger, 1988). CORINA is used to produce coordinates of an ideal 3-D conformation of each PDB ligand. MSDchem is an integral part of the Macromolecular Structure Search Database (MSDSD; Boutselakis et al., 2004) and is updated on a weekly basis with new and revised ligand definitions, resulting from significant curation and clean-up efforts by wwPDB. Many MSD and wwPDB tools reference this data (e.g., the Ligand Depot service described in *UNIT 1.9*).

The MSDchem search service offers various options for searching the ligand dictionary based on name, chemical formula, subgraph matching, or fingerprint similarity, as well as any combination of the above. While searching for a ligand using part of its code, name, synonym, or formula is useful in following literature or PDB file references, looking for molecules that contain a given chemical structure (subgraph searching) can be valuable when only an outline of the chemical diagram is known or when identifying variants of molecules that are expected to have similar chemical behavior in their common parts. On the other hand, chemical fingerprint similarity can be used to find ligands composed of a similar set of smaller subgroups, which may be connected differently but which have similar localized chemistry. Based on the results Web pages users may investigate, visualize, and export ligand structures or refer back to the relevant PDB entries. The MSDchem database is available for export in various formats: a ready-to-use relational database, collections of commonly used chemical data files, or SMILE string listings.

Contributed by Dimitris Dimitropoulos, John Ionides, and Kim Henrick
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Four protocols are included in this unit, the first of which covers the simplest search option where the three-letter code or a part of the molecular name is known (Basic Protocol 1). This is the most popular option because it provides an overview of the ligand details from a literature reference. The next two protocols cover searching using a molecular formula or chemical fragment (Basic Protocol 2) and subgraph matching (Basic Protocol 3). These types of searching provide increasingly more powerful and accurate options for interactive use of MSDchem. Basic Protocol 4, which involves exporting the ligand dictionary, is for users who need to apply their own tools and methods to a local copy of the data collection.

BASIC PROTOCOL 1

SEARCHING FOR LIGANDS USING THE THREE-LETTER PDB CODE OR MOLECULAR NAME

The most common reason for using MSDchem is to have a look at the chemical diagram and properties of a ligand mentioned in a PDB file or to search the literature by either its common three-letter PDB code or a chemical name. This protocol demonstrates how to use MSDchem to perform this fundamental task and familiarizes the user with the MSDchem Web pages.

Necessary Resources

Hardware

Computer with Internet access

Software

An up-to-date Internet browser, such as Internet Explorer 3.0 or later (*http://www.microsoft.com/ie*); Netscape 4.75 or later (*http://browser.netscape.com*); Firefox 1.0 or later (*http://www.mozilla.org/firefox*); or Safari (*http://www.apple.com/safari*)

Search for ligands using the three-letter PDB code

1. Open the MSDchem search home page (*http://www.ebi.ac.uk/msd-srv/msdchem*; Fig. 14.3.1).

   This page is the starting point for simple and advanced searches of the ligand dictionary with combinations of individual search constraints and access to export functionality and relevant documentation.

   The main area of the page provides version and summary information about the status of the database and the various text fields, controls, and buttons for selecting the search operators and invoking the constraint editors in order to build the value of constraints. Documentation about search fields can be found by following links from the data item labels (like "Molecule Name") or by using the adjacent question marks. There are various search operators for each search field that can be selected from the drop-down menu next to each search item name, and the most frequently used one is preselected.

   The top header area of the page provides Web links to the MSD group page at EBI, the MSD Web services toolbox, introductory MSDchem documentation, and e-mail address contact for feedback and questions. There is also a link for accessing the "Energy types" section of the MSDchem data that is used as a source for refinement dictionaries of crystallographic software packages (Krissinel et al., 2004) and a direct shortcut back to the MSDchem search home page.

   The left-hand menu area contains references to MSDchem guide, relevant literature and citations, and acknowledgments to software and resource contributors of MSDchem. This area has also links to alternative search pages and access to the ligand index and export pages.

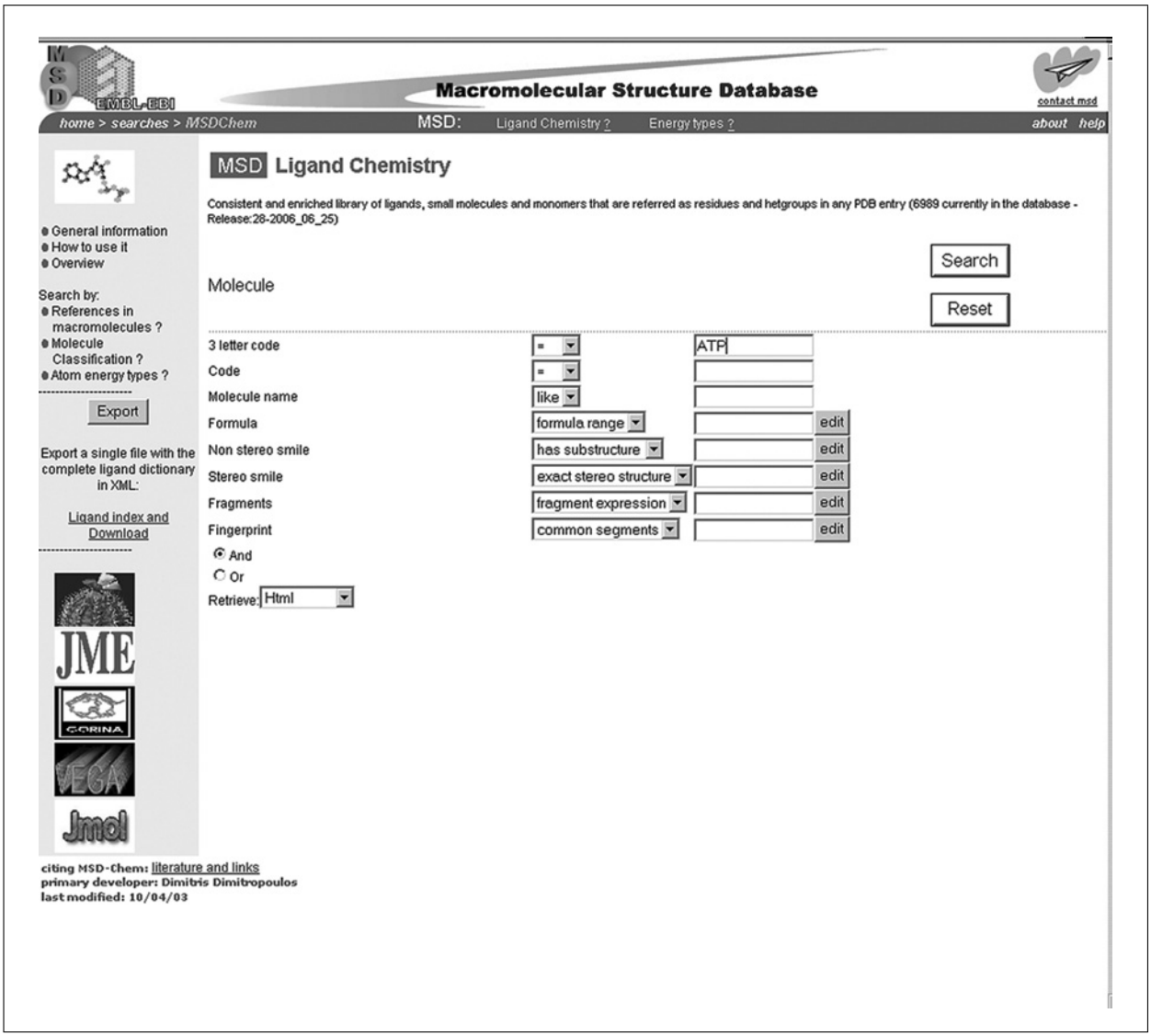


Figure 14.3.1 The MSDchem search home page. The figure illustrates how to find the ligand with a three-letter code of ATP.

2. Add the three-letter code or the name of the ligand of interest. For example, type `ATP` in the "3 letter code" text field.

   *The alternative Code text field is used when entering the MSD extended code, which in cases of topological variants can be different from the three-letter code. In the Molecule name text field, the user may input a part or a pattern of a molecular name. Both * and % are accepted as wildcard expressions (that match any number of characters). When no wildcards are used, they are automatically assumed at both ends, and searches are case insensitive. For example, the ligand MIT, with the common name ARGATROBAN, systematic name (2R,4R)-4-methyl-1-(N2-{[(3S)-3-methyl 1,2,3,4-tetrahydroquinolin-8-yl]sulfonyl}-L-arginyl)piperidine-2-carboxylic acid, and synonyms MD-805 and MITSUBISHI INHIBITOR will match all following molecule name expressions: inhibitor, *tetrahydroquinolin* acid, and ARGA%.*

3. Click on the Search button and view the list of ligand results. There is a row for each one of the ligands (in this example, only one) that match the search criteria in the result page, with summary details that include the three-letter code, the common name, and the formula, as well as a small overview image of its chemical drawing.

On the top of the page there are links to the list of PDB entries and binding site details (Golovin et al., 2005) for the set of these ligands.

Documentation can be found by following links from the data item names in the column headers just below the line reporting the number of results.

View a ligand details page

4. Click on the three-letter code (the PDB reference) to navigate to an individual ligand details page. The resulting page is shown in Figure 14.3.2. In this page there is extensive data about the molecule, e.g., common and systematic names, stereo and nonstereo SMILE strings, formula and molecular charge, and number of total and heavy (non-hydrogen) atoms. There is also a larger chemical diagram of the molecule with atoms identified by their common PDB atom names. This diagram and other information in this page provide an understanding of the chemical context of the atoms observed in the PDB experiment. Atoms are colored based on their element type, bond orders, and stereo configurations, while aromatic bonds are displayed using gray instead of the black color used for other bond types.

5. Click on the Atoms link on the left hand side of the page to obtain more detailed data. The view shown in Figure 14.3.3 appears, providing a list of the atoms of the ligand with explicit stereodescriptors, aromatic flags, atomic charges, idealized 3-D coordinates, and other data at the atomic level.

   Additionally, one may get a summary page with all the entities that are part of or associated with the ligand using the Contents or the Complete contents links.

   As usual, the documentation for all data items in all pages is available from links accessed by clicking on the data item names or the close-by question marks.

Visualize the ligand in three dimensions

6. Select a coordinate set from the Library drop-down menu on the left side of the ligand details page (Fig. 14.3.2) by choosing one of the menu items described below.

   Ideal: idealized 3-D coordinates that are generated automatically by the CORINA (Gasteiger et al., 1990) software package. CORINA does not use experimental data but only the molecule connectivity, bond orders, and chirality to produce a conformation of the molecule that is energetically favorable in isolation and visually elegant in the 3-D space.

   PDB: the set of representative coordinates that wwPDB curators have manually chosen from all the occurrences of the ligand in PDB files. The "PDB representative conformation" of a ligand is chosen from the PDB file of an experiment with the best possible resolution after the curators make sure that there are no errors or conflicts with this coordinate set and the chemical structure of the ligand as given by its chemical diagram. This conformation is the result of the interaction of the ligand with a protein and is useful in understanding its biological function.

   The other menu option, PDB+H (representative non-hydrogen atom coordinates with idealized hydrogen coordinates), is not used in this step since hydrogen atoms are not visible for reasons of clarity.

7. Select the viewer of preference from the Viewers drop down menu by choosing one of the following:

   Jmol applet viewer, which will work with any browser without any other prerequisites, but is missing some functionality of other popular viewers, or

   Rasmol/rastop variant viewer, which must be installed by the user.

   This viewer must be configured as the "chemical/x-pdb" mime type handler of the computer, associated with `.pdb` *files.*

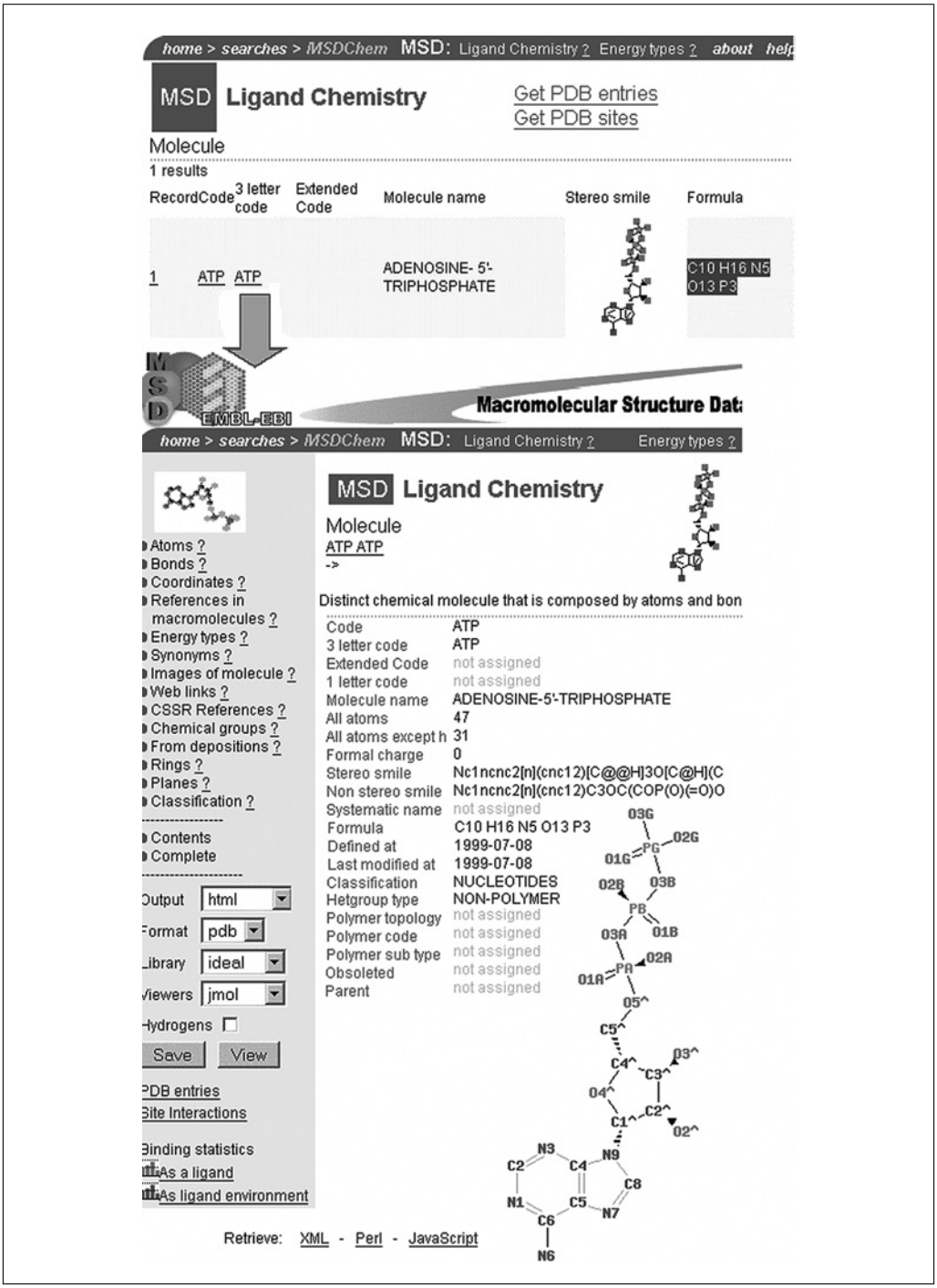
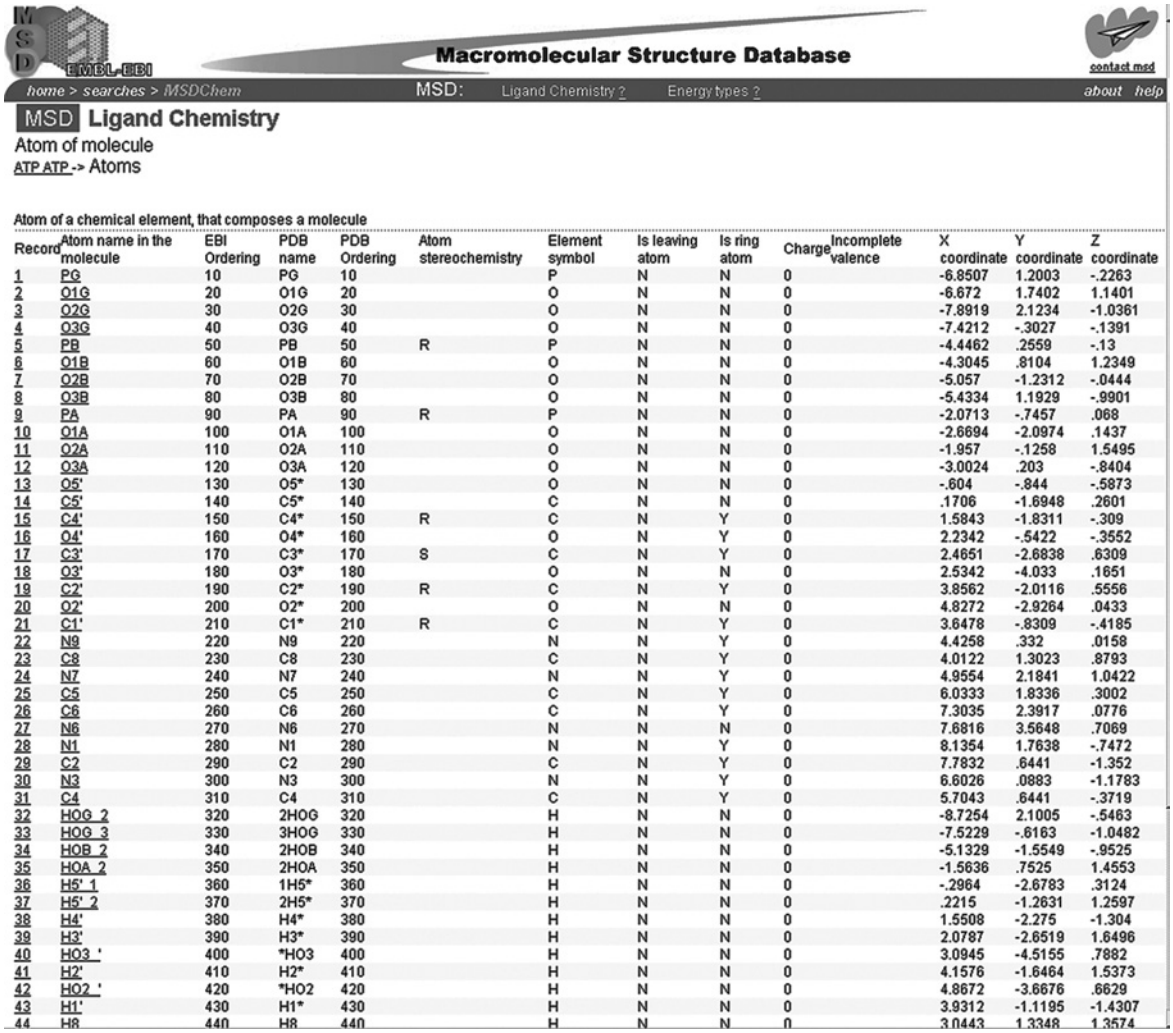


Figure 14.3.2 The MSDchem result page (top), listing the ligand with the three-letter code of ATP that matches the search criteria, and the ligand details page (bottom) with information about the ligand properties. Links to ligand content and related data, visualization and export functionality, and the PDB nomenclature chemical diagram are provided.

8. Click on the View button to obtain one of the views (idealized or representative) shown in Figure 14.3.4.

Export ligand data in different file formats

9. Select sdf from the Format drop down menu on the left side of the ligand details page (Fig. 14.3.2).



Macromolecular Structure Database

home > searches > MSDChem MSD: Ligand Chemistry ? Energy types ? about help

contact msd

MSD Ligand Chemistry

Atom of molecule

ATP ATP -> Atoms

Atom of a chemical element, that composes a molecule

Record	Atom name in the molecule	EBI Ordering	PDB name	PDB Ordering	Atom stereochemistry	Element symbol	Is leaving atom	Is ring atom	Charge	Incomplete valence	X coordinate	Y coordinate	Z coordinate
1	PG	10	PG	10		P	N	N	0		-6.8507	1.2003	-.2263
2	O1G	20	O1G	20		O	N	N	0		-6.672	1.7402	1.1401
3	O2G	30	O2G	30		O	N	N	0		-7.8919	2.1234	-1.0361
4	O3G	40	O3G	40		O	N	N	0		-7.4212	-.3027	-.1391
5	PB	50	PB	50	R	P	N	N	0		-4.4462	.2559	-.13
6	O1B	60	O1B	60		O	N	N	0		-4.3045	.8104	1.2349
7	O2B	70	O2B	70		O	N	N	0		-5.057	-1.2312	-.0444
8	O3B	80	O3B	80		O	N	N	0		-5.4334	1.1929	-.9901
9	PA	90	PA	90	R	P	N	N	0		-2.0713	-.7457	.068
10	O1A	100	O1A	100		O	N	N	0		-2.6694	-2.0974	.1437
11	O2A	110	O2A	110		O	N	N	0		-1.957	-.1258	1.5495
12	O3A	120	O3A	120		O	N	N	0		-3.0024	.203	-.8404
13	O5'	130	O5*	130		O	N	N	0		-.604	-.844	-.5873
14	C5'	140	C5*	140		C	N	N	0		.1706	-1.6948	.2601
15	C4'	150	C4*	150	R	C	N	Y	0		1.5843	-1.8311	-.309
16	O4'	160	O4*	160		O	N	Y	0		2.2342	-.5422	-.3552
17	C3'	170	C3*	170	S	C	N	Y	0		2.4651	-2.6838	.6309
18	O3'	180	O3*	180		O	N	N	0		2.5342	-4.033	.1651
19	C2'	190	C2*	190	R	C	N	Y	0		3.8562	-2.0116	.5556
20	O2'	200	O2*	200		O	N	N	0		4.8272	-2.9264	.0433
21	C1'	210	C1*	210	R	C	N	Y	0		3.6478	-.8309	-.4185
22	N9	220	N9	220		N	N	Y	0		4.4258	.332	.0158
23	C8	230	C8	230		C	N	Y	0		4.0122	1.3023	.8793
24	N7	240	N7	240		N	N	Y	0		4.9554	2.1841	1.0422
25	C5	250	C5	250		C	N	Y	0		6.0333	1.8336	.3002
26	C6	260	C6	260		C	N	Y	0		7.3035	2.3917	.0776
27	N6	270	N6	270		N	N	N	0		7.6816	3.5648	.7069
28	N1	280	N1	280		N	N	Y	0		8.1354	1.7638	-.7472
29	C2	290	C2	290		C	N	Y	0		7.7832	.6441	-1.352
30	N3	300	N3	300		N	N	Y	0		6.6026	.0883	-1.1783
31	C4	310	C4	310		C	N	Y	0		5.7043	.6441	-.3719
32	HOG_2	320	2HOG	320		H	N	N	0		-8.7254	2.1005	-.5463
33	HOG_3	330	3HOG	330		H	N	N	0		-7.5229	-.6163	-1.0482
34	HOB_2	340	2HOB	340		H	N	N	0		-5.1329	-1.5549	-.9525
35	HOA_2	350	2HOA	350		H	N	N	0		-1.5636	.7525	1.4553
36	H5'_1	360	1H5*	360		H	N	N	0		-.2964	-2.6783	.3124
37	H5'_2	370	2H5*	370		H	N	N	0		.2215	-1.2631	1.2597
38	H4'	380	H4*	380		H	N	N	0		1.5508	-2.275	-1.304
39	H3'	390	H3*	390		H	N	N	0		2.0787	-2.6519	1.6496
40	HO3_'	400	*HO3	400		H	N	N	0		3.0945	-4.5155	.7882
41	H2'	410	H2*	410		H	N	N	0		4.1576	-1.6464	1.5373
42	HO2_'	420	*HO2	420		H	N	N	0		4.8672	-3.6676	.6629
43	H1'	430	H1*	430		H	N	N	0		3.9312	-1.1195	-1.4307
44	H8	440	H8	440		H	N	N	0		3.0443	1.3348	1.3574

Figure 14.3.3 MSDchem ligand data at the atomic level that can be accessed from a ligand details page.

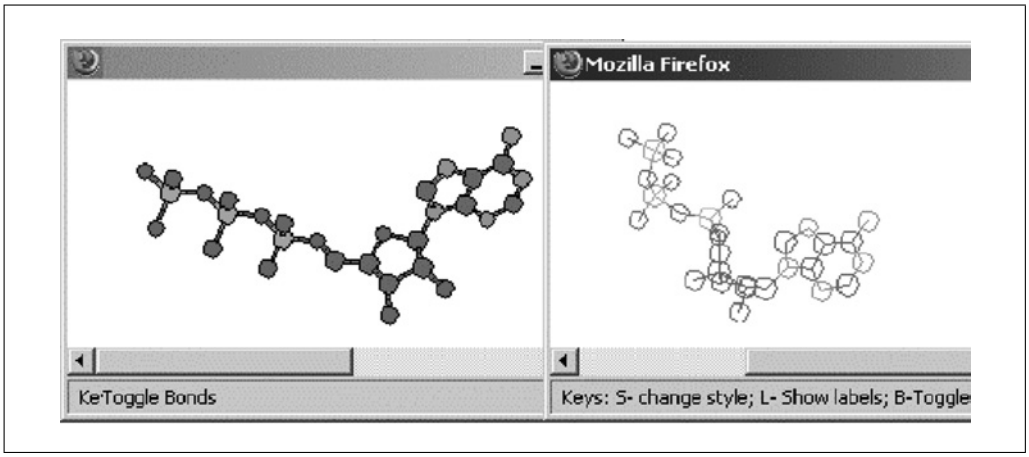


Figure 14.3.4 Three-dimensional visualizations of a ligand using the Jmol applet for idealized versus representative coordinates from MSDchem.

Other choices include PDB, crystallographic mmCif, CML (Chemical Mark-up Language), and XYZ file formats. The reason that MSDChem offers all these alternative export formats is that the most common ones are not designed to store every important piece of information. PDB ligand export files can be easily incorporated and used in parallel with files from the actual PDB archive but are missing placeholders for important chemical properties like bond orders. SDF/MDL format, on the other hand, is one of the most popular formats in chemoinformatics in that it is able to store the definitive chemical properties. However, it has no place for PDB atom labels, which are used to provide direct literature references for ligands on the atom level. Crystallographic mmCif is the format of the wwPDB exchange designed to solve the problems of incomplete ligand representation but is not been widely used by chemical and visualization software. CML is an XML-based format ideal for programmatic use and slowly gaining in popularity, while XYZ is a very primitive format supported by various general purpose 3-D visualization packages.

10. Select the HTML option from the Output drop down menu.

    Other choices allow a user the options to either download the file on the hard disk to later open using a text editor or another program or have a look at the contents of the file directly on a separate browser window.

11. From the Library drop down menu choose either ideal (to use idealized) or PDB (to use representative coordinates). The Hydrogens checkbox specifies whether to include hydrogen atoms in the exported files; uncheck this option in order to exclude hydrogens.

    Files that include hydrogen atoms provide a more complete data set, but excluding the hydrogen atoms may often simplify visualization and processing of the really significant chemical structure of the heavier atoms. If hydrogen atoms are required, use the pdb+H option from the Library menu to get representative PDB heavy atom coordinates together with CACTVS (Ihlenfeldt et al., 1992) idealized hydrogen coordinates. This is usually a good idea since hydrogen coordinates are often missing from the PDB, and the hydrogen atoms will have null (zero) coordinates in the exported file. Most export formats do not distinguish between an atom in the three-dimensional point (0,0,0) from an unobserved atom, with unpredictable results.

12. Click on the Save button. The pop-up window shown in Figure 14.3.5 will appear.

13. Access the PDB entries that include the ligand, and their binding site information.

    On the bottom of the left menu area of the ligand details page, there are links that redirect the user to the list of PDB entries containing the ligand from MSDlite (Golovin et al.,

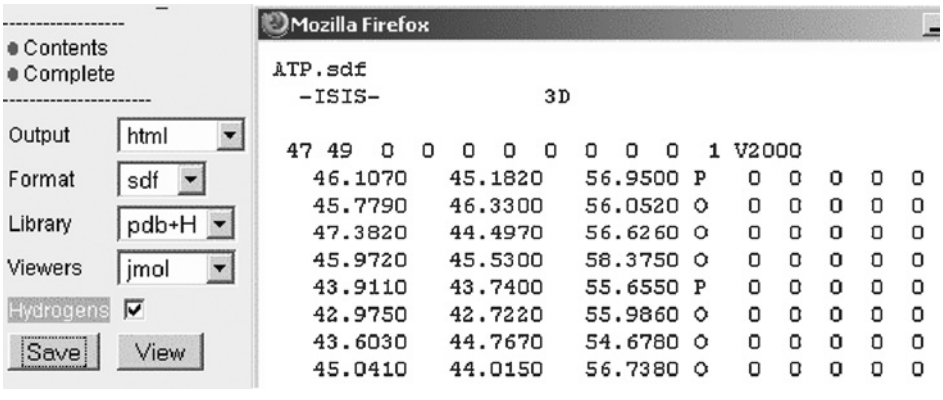


Figure 14.3.5 Exporting ligand data with representative heavy-atom and idealized hydrogen coordinates in the SDF/MDL chemical file format using MSDchem.

2004), as well as to details about its binding sites from MSDsite (Golovin et al., 2005). For example, following the link for binding statistics labeled "As a Ligand" produces a chart with the relative frequencies of interactions with various amino acids. The next link below, labeled "As ligand environment," is useful for standard and modified amino acids that can be part of a bound molecule's environment.

BASIC PROTOCOL 2

SEARCHING FOR LIGANDS USING A FORMULA OR FRAGMENT EXPRESSION

Remembering ligand three-letter codes and avoiding mistakes in spelling molecular names are not easy. Additionally, it may be desirable to perform searches that do not target a single ligand, but rather a class of ligands that share some common chemical characteristics (e.g., atoms of particular elements or common chemical groups). A simple way of performing this type of search is by using a formula range expression or a pharmacophore fragment expression. Following the steps of this protocol will facilitate converting these elements into a formula or fragment-based search option that may significantly reduce the number of candidate ligands that have to be inspected.

Necessary Resources

Hardware

Computer with Internet access

Software

An up-to-date Internet browser, such as Internet Explorer 3.0 or later (*http://www.microsoft.com/ie*); Netscape 4.75 or later (*http://browser.netscape.com*); Firefox 1.0 or later (*http://www.mozilla.org/firefox*)

Use the formula expression editor

1. Open the MSDchem search home page (*http://www.ebi.ac.uk/msd-srv/msdchem*; Fig. 14.3.1)
2. Enter a formula range expression (a space-separated list of chemical elements followed by a value or a range for the number of times this element is allowed in the ligand formula).

   The syntax for a formula range expression is as follows.

   [<Element> | <Element><Value> | <Element><Minimum>–<Maximum>]

   A range has to be given in the form of "minimum value"-"maximum value" (separated with the "-" character). Elements given without a specified value or a range are equivalent to <Element>1 while <Element>0 means that the particular element is not allowed at all. Elements not given in the expression at all may or may not be part of the ligand formula.

   For example: C3-6 N2 F (three to six carbon atoms, exactly two nitrogen atoms, a fluorine, and anything else); O1-4 N3-100 CL1-100 F0 S0 (no more than four oxygen atoms, at least three nitrogen atoms, at least one chlorine, no fluorine or sulfur, and anything else).

3. Alternatively, click on the "edit" button on the same line as the Formula text field to bring up the formula expression editor window shown in Figure 14.3.6.

   Using the formula expression editor is optional, but it is a fast and easy way to build an expression in an interactive way, without having to worry that the formula range expression being queried is incorrect.

4. If using the formula expression editor window, leave the default "formula range" search operator selected and specify, for example, ligands with one to four oxygen

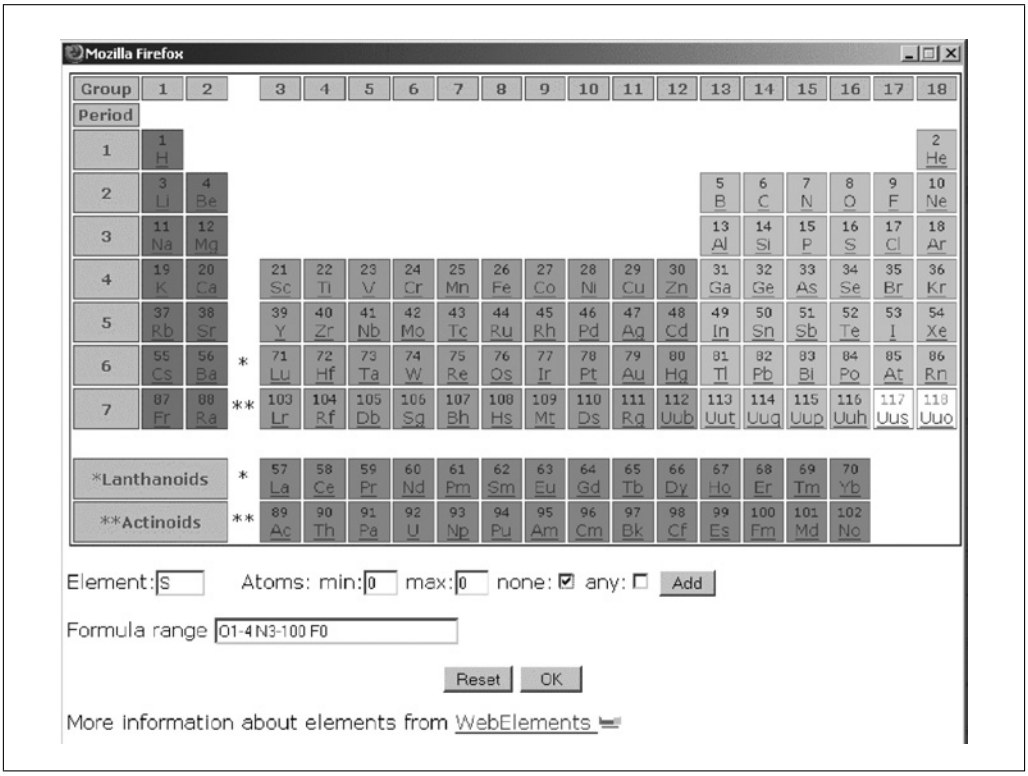

Mozilla Firefox
Element: S
Atoms: min: 0 max: 0 none: any: Add
Formula range O1-4 N3-100 F0
Reset OK
More information about elements from WebElements

Figure 14.3.6 The formula expression editor screen used in an example to obtain MSDchem ligands with one to four oxygen atoms, at least three nitrogen atoms, no fluorine, and no sulfur.

atoms, more than three nitrogen atoms, and no fluorine or sulfur, by performing the following steps:

a. Click on O (oxygen) and type in `1` and `4` as the min and max values. Click on Add.

b. Click on N (nitrogen) and specify `3` as the min value. Click on Add.

c. Click on F (fluorine) and tick the "none" checkbox. Click on Add.

d. Repeat the same step for S (sulfur).

e. When finished, click on OK to transfer the expression in the search page.

In order to add a constraint for a new element on the formula, first click on the corresponding element, choose either the "any" or "none" check box, or alternatively provide input values into the min and max fields and click on the Add button to append the new constraint in the formula expression. Just clicking on an element name and immediately on the OK button will generate the expression equivalent to "any number of atoms of this element" (the value of 100 is realistically a number for no actual upper limit).

Use the fragment expression editor

5. Go to the MSDchem search home page (*http://www.ebi.ac.uk/msd-srv/msdchem*). Click on the "edit" button that is on the same line as the Fragments text field to bring up the fragment expression editor window shown in Figure 14.3.7. Enter a fragment name, followed by a value or a range, for the number of times this fragment is allowed in the ligand formula, as in the following formal description:

[<Fragment> | <Fragment><Value> | <Fragment><Min>-<Max>]

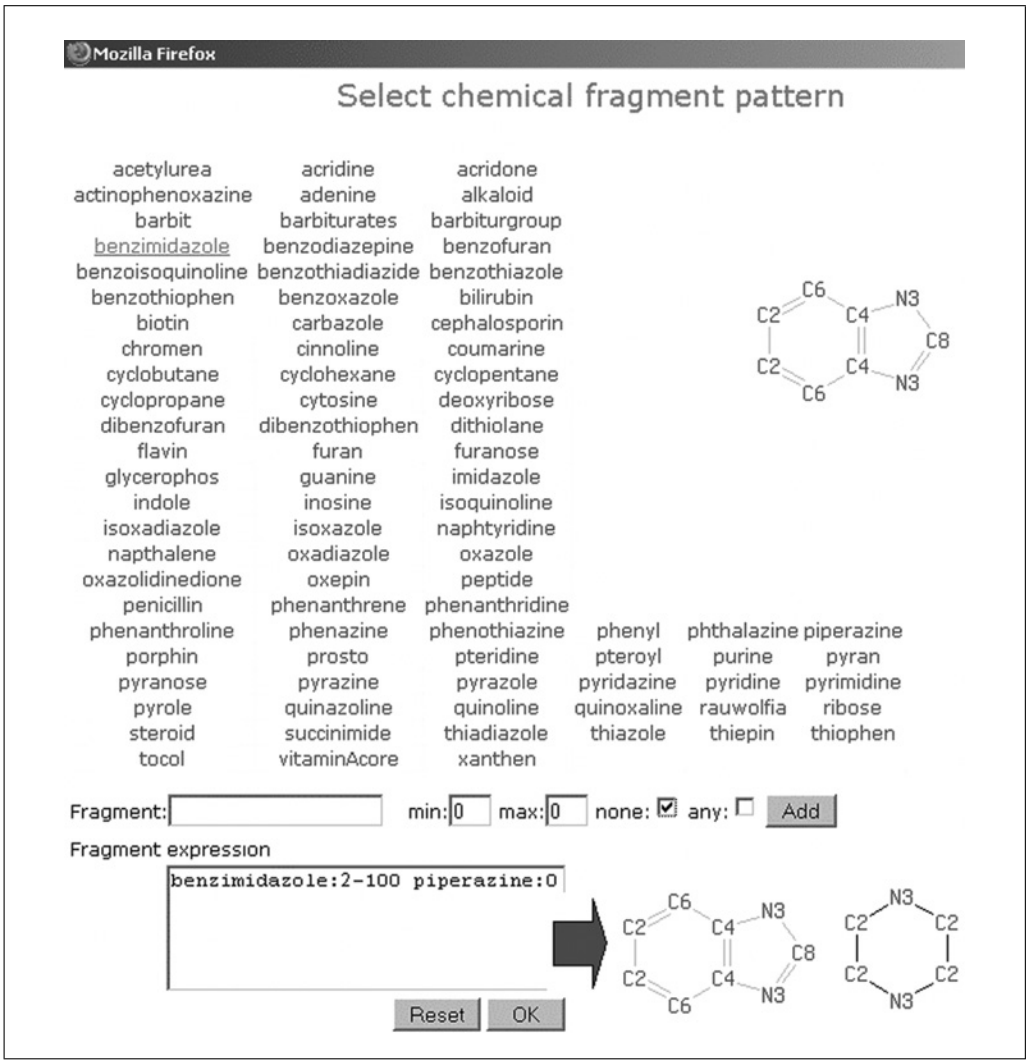


Figure 14.3.7 The fragment expression editor screen used in an example to obtain MSDchem ligands with two or more benzimidazole and without any piperazine groups.

The benzimidazole and piperazine groups used are shown Figure 14.3.7. There are fragments for about 90 common functional groups that are chosen to be large and characteristic enough to locate real pharmacophores. The selection, which is based on published literature, is expected to be revised in the future.

Using the fragment editor is convenient because it has a context sensitive list of various predefined fragments. Whenever the mouse cursor is moved above a group name, the corresponding fragment is displayed on the top right area of the editor.

Fragments and their display images often include wildcard "green colored - X" types for atom elements and wildcard "green colored - any" orders for bonds. Aromatic bonds are displayed with gray color. In order to add a constraint for a new fragment click on the corresponding group name, choose either the "any" or "none" check box. Alternatively, provide input values into the "min"-"max" field (for the number of times the fragment should appear in the molecule).

6. For example, use the fragment expression editor in building an expression for ligands that have at least two benzimidazole and no piperazine groups by performing the following steps:

   a. Click on the benzimidazole group and specify `2` in the min text field.

   b. Click on the Add button to specify at least two benzimidazole groups.

c. Click on the piperazine group and tick the "none" check box.

d. Click on the Add button and then (when finished with all fragments) click on the OK button. The search home page shown in Figure 14.3.1 will appear, with the search fields filled out according to the selection made in the previous step.

7. Click on the Search button on the search page to get the list of PDB ligands with one to four oxygen atoms, at least three nitrogen atoms, no fluorine or sulfur, at least two benzimidazole groups, and no piperazine groups to obtain the view shown in Figure 14.3.8.

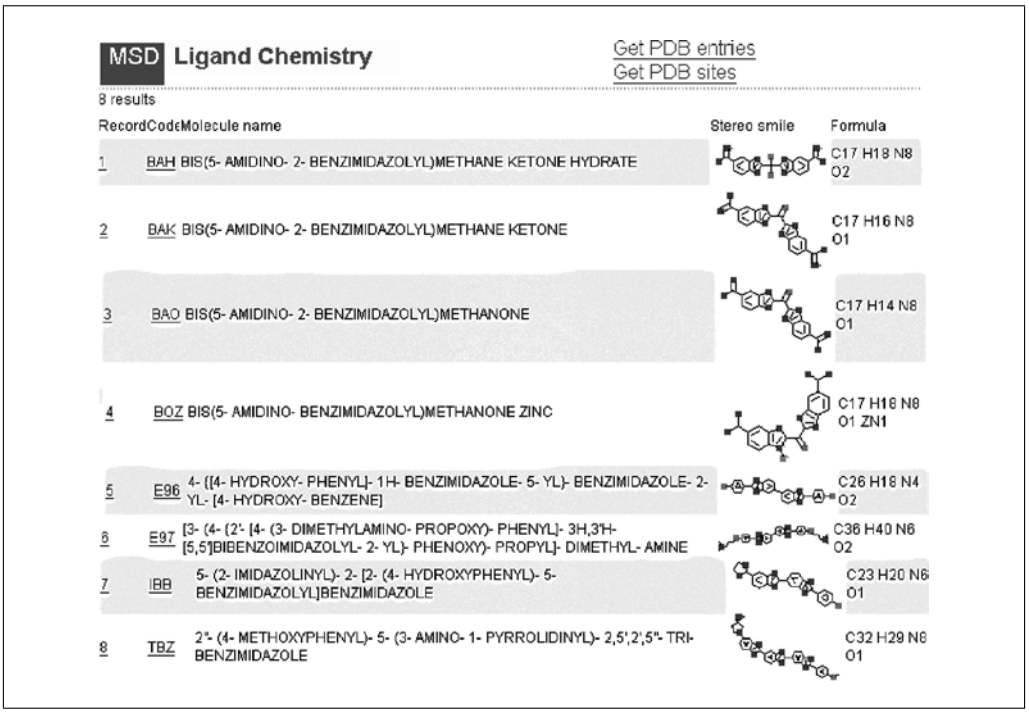

MSD Ligand Chemistry
Get PDB entries
Get PDB sites
8 results
RecordCodeMolecule name
Stereo smile
Formula
1 BAH BIS(5- AMIDINO- 2- BENZIMIDAZOLYL)METHANE KETONE HYDRATE C17 H18 N8 O2
2 BAK BIS(5- AMIDINO- 2- BENZIMIDAZOLYL)METHANE KETONE C17 H16 N8 O1
3 BAO BIS(5- AMIDINO- 2- BENZIMIDAZOLYL)METHANONE C17 H14 N8 O1
4 BOZ BIS(5- AMIDINO- BENZIMIDAZOLYL)METHANONE ZINC C17 H18 N8 O1 ZN1
5 E96 4- ([4- HYDROXY- PHENYL]- 1H- BENZIMIDAZOLE- 5- YL)- BENZIMIDAZOLE- 2- YL- [4- HYDROXY- BENZENE] C26 H18 N4 O2
6 E97 [3- (4- (2'- [4- (3- DIMETHYLAMINO- PROPOXY)- PHENYL]- 3H,3'H- [5,5']BIBENZOIMIDAZOLYL- 2- YL)- PHENOXY)- PROPYL]- DIMETHYL- AMINE C36 H40 N6 O2
7 IBB 5- (2- IMIDAZOLINYL)- 2- [2- (4- HYDROXYPHENYL)- 5- BENZIMIDAZOLYL]BENZIMIDAZOLE C23 H20 N6 O1
8 TBZ 2'- (4- METHOXYPHENYL)- 5- (3- AMINO- 1- PYRROLIDINYL)- 2,5',2',5''- TRI- BENZIMIDAZOLE C32 H29 N8 O1

Figure 14.3.8 MSDchem ligands that satisfy particular formula range and fragment expression constraints.

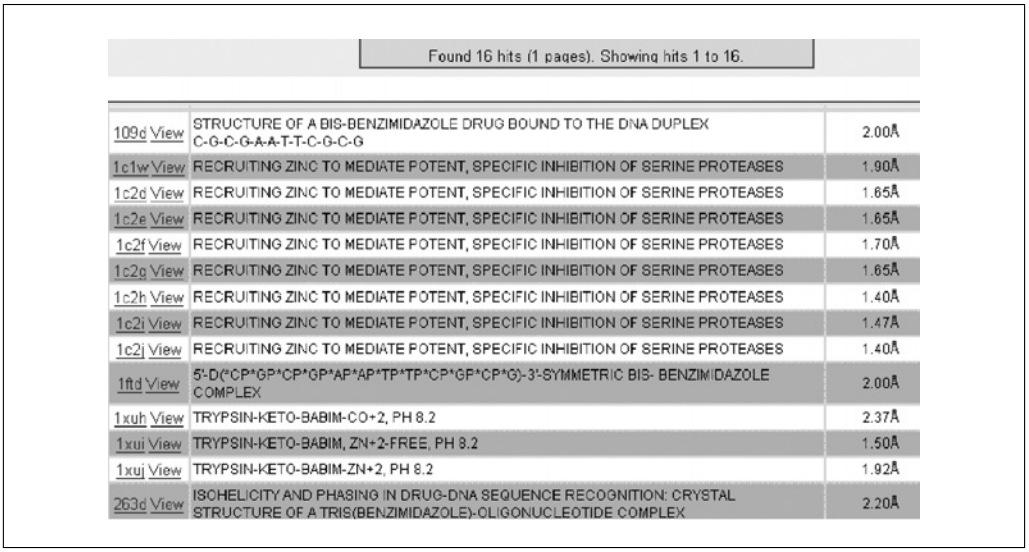

Found 16 hits (1 pages). Showing hits 1 to 16.
109d View STRUCTURE OF A BIS-BENZIMIDAZOLE DRUG BOUND TO THE DNA DUPLEX C-G-C-G-A-A-T-T-C-G-C-G 2.00Å
1c1w View RECRUITING ZINC TO MEDIATE POTENT, SPECIFIC INHIBITION OF SERINE PROTEASES 1.90Å
1c2d View RECRUITING ZINC TO MEDIATE POTENT, SPECIFIC INHIBITION OF SERINE PROTEASES 1.65Å
1c2e View RECRUITING ZINC TO MEDIATE POTENT, SPECIFIC INHIBITION OF SERINE PROTEASES 1.65Å
1c2f View RECRUITING ZINC TO MEDIATE POTENT, SPECIFIC INHIBITION OF SERINE PROTEASES 1.70Å
1c2g View RECRUITING ZINC TO MEDIATE POTENT, SPECIFIC INHIBITION OF SERINE PROTEASES 1.65Å
1c2h View RECRUITING ZINC TO MEDIATE POTENT, SPECIFIC INHIBITION OF SERINE PROTEASES 1.40Å
1c2i View RECRUITING ZINC TO MEDIATE POTENT, SPECIFIC INHIBITION OF SERINE PROTEASES 1.47Å
1c2j View RECRUITING ZINC TO MEDIATE POTENT, SPECIFIC INHIBITION OF SERINE PROTEASES 1.40Å
1ftd View 5'-D(*CP*GP*CP*GP*AP*AP*TP*TP*CP*GP*CP*G)-3'-SYMMETRIC BIS- BENZIMIDAZOLE COMPLEX 2.00Å
1xuh View TRYPSIN-KETO-BABIM-CO+2, PH 8.2 2.37Å
1xui View TRYPSIN-KETO-BABIM, ZN+2-FREE, PH 8.2 1.50Å
1xuj View TRYPSIN-KETO-BABIM-ZN+2, PH 8.2 1.92Å
263d View ISOHELICITY AND PHASING IN DRUG-DNA SEQUENCE RECOGNITION: CRYSTAL STRUCTURE OF A TRIS(BENZIMIDAZOLE)-OLIGONUCLEOTIDE COMPLEX 2.20Å

Figure 14.3.9 List of PDB entries referring to MSD atlas pages that include ligands that satisfy particular formula range and fragment expression constraints.

8. Follow links in the results list for details about each individual ligand using the same steps explained in Basic Protocol 1.
9. Obtain a list of PDB entries from the links on the top of the page for information on where these ligands can be found or about their binding site details (e.g., see Fig. 14.3.9).
10. Follow the four-character PDB file name links in the list of PDB entries to the atlas pages that provide summary information about the PDB entries, or follow the "view" links to activate a protein 3-D visualization applet for the whole PDB entry.

BASIC PROTOCOL 3

PERFORMING A CHEMICAL SUBGRAPH SEARCH

Formula and chemical fragment searching is appropriate in cases where little is known about the ligand chemical structure. Often one may not remember the three-letter PDB code or chemical name of a ligand but may still easily draw up the diagram of a significant part of its chemical structure. In cases where the connectivity diagram for a reasonable fraction of the molecule is known or there is a ligand that is quite similar in terms of chemical structure, the steps in this protocol may be used to search for a chemical subgraph, i.e., a subset of the atoms and bond of the target ligand drawn up in a chemical diagram editor. This procedure will return a restricted list of more accurate candidate molecules that include the input chemical structure, and it can be also used to look for ligand variants.

A convenient and popular way to encode a chemical structure into a text string is by using SMILE strings that are equivalent to chemical formulas but also incorporate the atom connectivity and chemical properties. A nonstereo SMILE will encode all the fundamental information found on a chemical diagram but with unspecified atom chirality. This information is about atom elements, formal charges and connectivity, as well as bond orders. Nonstereo SMILEs will not be able to distinguish between two different stereo-isomers, while stereo SMILEs, which also encode stereo descriptors of atoms and bonds, will. It is usually a lot more convenient to use the nonstereo SMILEs as the search criteria and then to visually inspect all the stereoisomers. Reading and writing SMILE strings is a rather difficult exercise for larger molecules and this where a molecular editor will definitely help.

Necessary Resources

Hardware

Computer with Internet access

Software

An up-to-date Internet browser, such as Internet Explorer 3.0 or later (*http://www.microsoft.com/ie*); Netscape 4.75 or later (*http://browser.netscape.com*); Firefox 1.0 or later (*http://www.mozilla.org/firefox*)

1. Open the MSDchem search home page (*http://www.ebi.ac.uk/msd-srv/msdchem*; Fig. 14.3.1).
2. Open the JME molecular editor by clicking on the "edit" button on the same line as the "Non stereo smile" text field. Sketch the ligand structure or modify the ligand structure of a similar known ligand (Fig. 14.3.10).

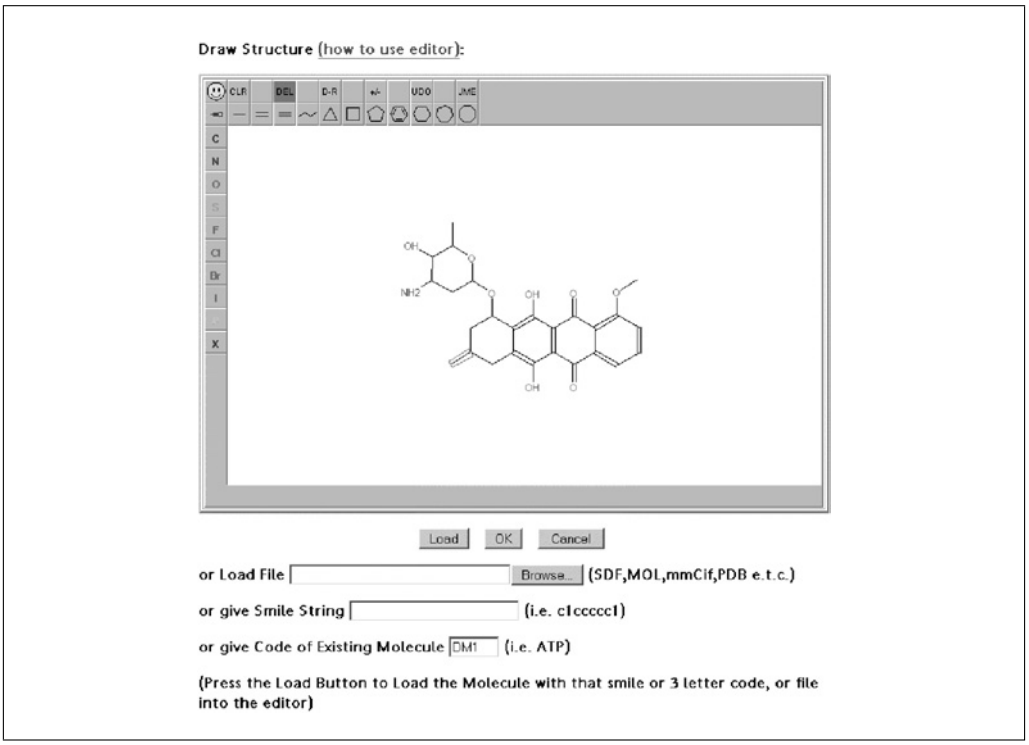


Figure 14.3.10 Screen used for loading the molecular structure diagram of DM1 on JME editor and modifying it by removing its noncharacteristic atoms or groups in order to prepare a subgraph search criteria that will match molecules with the same main structure as DM1.

3. For example, to load the chemical diagram of daunomycin, type the three-letter code (`DM1` in this case), a chemical file name (SDF/MOL, mmCif, PDB), or a SMILE string into the appropriate field on the molecular editor page and click Load. The molecular diagram of DM1 will appear on the JME editor.

   The JME molecular editor is a Java applet embedded in a Web page that offers the functionality of drawing a chemical diagram. It has controls to add bonds and small groups (common rings) on the molecule, modify existing atom elements and bond orders, and remove bonds together with their atoms. In order to use it, first sketch the connectivity diagram of the desired substructure and then finalize it by modifying noncarbon heavy elements and bond orders. Use of hydrogen atoms is not recommended, and the editor will not allow input of a disconnected chemical graph.

4. Click on the delete (DEL) button of JME, and then click on all bonds of the OH and C-C=O groups linked to the C12 ring atom on the left bottom of the structure to remove the hydroxyl and methylCO nonring groups linked to atom C12.

5. Click on the OK button to transfer the SMILE string of this chemical subgraph on the search page.

   Alternatively, skip using the molecular editor altogether by directly inputting the SMILE string or a three-letter code that will be used as a subgraph, assuming that these groups are not characteristic for the substructure being searched in this example. The result will be a more generalized chemical subgraph of DM1.

6. Leave the default "has substructure" search operator selected.

7. Click on Search to get the list of molecules that include the substructure. The partial result is shown in Figure 14.3.11. There are ten ligands that match the specified subgraph search criteria. The images in the results list indicate that they are very similar

MSD **Ligand Chemistry**

Get PDB entries
Get PDB sites

Molecule

10 results

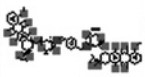
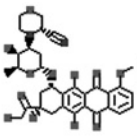
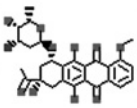
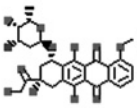
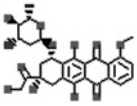
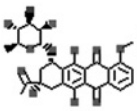
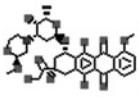
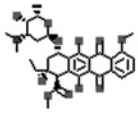
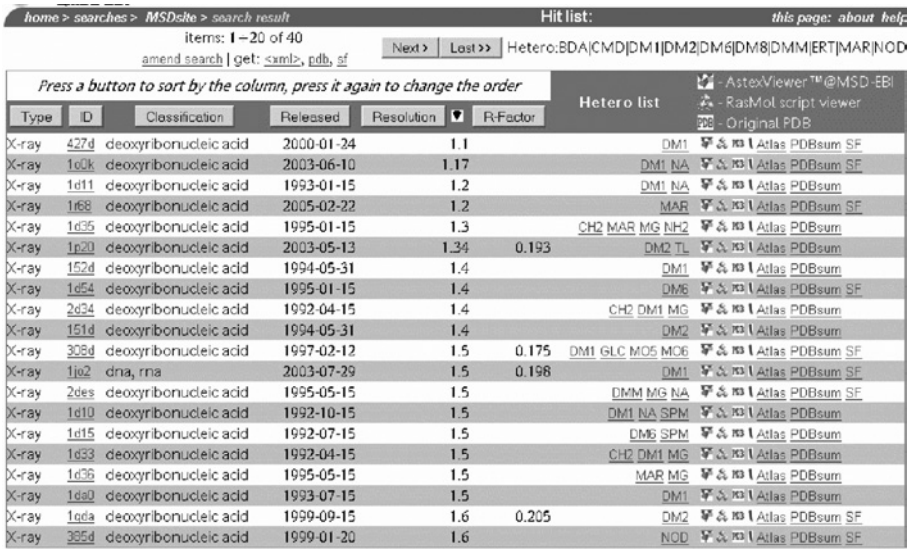
Record	Code	3 letter code	Extended Code	Molecule name	Stereo smile	Formula
1	BDA	BDA		4- METHYLBENZYL- N- BIS[DAUNOMYCIN]		C62 H66 N2 O20
2	CMD	CMD		3'- DESAMINO- 3'- (3- CYANO- 4- MORPHOLINYL)- DOXORUBICIN		C32 H34 N2 O12
3	DM1	DM1		DAUNOMYCIN		C27 H29 N1 O10
4	DM2	DM2		DOXORUBICIN		C27 H29 N1 O11
5	DM6	DM6		4'- EPIDOXORUBICIN		C27 H30 N1 O11
6	DM8	DM8		2'- BROMO- 4'- EPIDAUNORUBICIN		C27 H28 N1 O10 BR1
7	DMM	DMM		3'- DESAMINO- 3'- (2- METHOXY- 4- MORPHOLINYL)- DOXORUBICIN		C32 H37 N1 O13
8	ERT	ERT		METHYL (4R)- 2- ETHYL- 2,5,12- TRIHYDROXY- 7- METHOXY- 6,11- DIOXO- 4- {[2,3,6- TRIDEOXY- 3- (DIMETHYLAMINO)- BETA- D- RIBO- HEXOPYRANOSYL]OXY}- 1H,2H,3H,4H,6H,11H- TETRACENE- 1- CARBOXYLATE		C31 H37 N1 O11

Figure 14.3.11 Eight of the ten daunomycin-like ligands that contain the reduced chemical graph of DM1 as a subgraph, retrieved using the MSDchem "has substructure" search functionality.

molecules, and even the molecular names for most of them suggest that they fall in the class of daunomycin/doxorubicin variants.

In the case of ERT, the molecule name does not resemble anything, and this is an obvious example where only a subgraph search will identify the similarity of this ligand with daunomycins and doxorubicins while a name based one would not.

Subgraph searching is nontrivial problem, and this means that often the results are not instantaneous. The user is warned by a pop-up window that the search may require a couple of minutes to finish, but in practice, in the majority of cases, the results are available a lot faster.



home > searches > MSDsite > search result — Hit list: — this page: about help

items: 1 – 20 of 40 — Next > | Last >> — Hetero:BDA|CMD|DM1|DM2|DM6|DM8|DMM|ERT|MAR|NOD

amend search | get: <xml>, pdb, sf

Press a button to sort by the column, press it again to change the order

Type	ID	Classification	Released	Resolution	R-Factor	Hetero list	- AstexViewer™@MSD-EBI / - RasMol script viewer / PDB - Original PDB
X-ray	427d	deoxyribonucleic acid	2000-01-24	1.1		DM1	Atlas PDBsum SF
X-ray	1o0k	deoxyribonucleic acid	2003-06-10	1.17		DM1 NA	Atlas PDBsum SF
X-ray	1d11	deoxyribonucleic acid	1993-01-15	1.2		DM1 NA	Atlas PDBsum
X-ray	1f68	deoxyribonucleic acid	2005-02-22	1.2		MAR	Atlas PDBsum SF
X-ray	1d95	deoxyribonucleic acid	1995-01-15	1.3		CH2 MAR MG NH2	Atlas PDBsum
X-ray	1p20	deoxyribonucleic acid	2003-05-13	1.34	0.193	DM2 TL	Atlas PDBsum
X-ray	152d	deoxyribonucleic acid	1994-05-31	1.4		DM1	Atlas PDBsum
X-ray	1d54	deoxyribonucleic acid	1995-01-15	1.4		DM6	Atlas PDBsum SF
X-ray	2d34	deoxyribonucleic acid	1992-04-15	1.4		CH2 DM1 MG	Atlas PDBsum
X-ray	151d	deoxyribonucleic acid	1994-05-31	1.4		DM2	Atlas PDBsum
X-ray	308d	deoxyribonucleic acid	1997-02-12	1.5	0.175	DM1 GLC MO5 MO6	Atlas PDBsum SF
X-ray	1jo2	dna, rna	2003-07-29	1.5	0.198	DM1	Atlas PDBsum SF
X-ray	2des	deoxyribonucleic acid	1995-05-15	1.5		DMM MG NA	Atlas PDBsum SF
X-ray	1d10	deoxyribonucleic acid	1992-10-15	1.5		DM1 NA SPM	Atlas PDBsum
X-ray	1d15	deoxyribonucleic acid	1992-07-15	1.5		DM6 SPM	Atlas PDBsum
X-ray	1d33	deoxyribonucleic acid	1992-04-15	1.5		CH2 DM1 MG	Atlas PDBsum
X-ray	1d36	deoxyribonucleic acid	1995-05-15	1.5		MAR MG	Atlas PDBsum
X-ray	1da0	deoxyribonucleic acid	1993-07-15	1.5		DM1	Atlas PDBsum
X-ray	1qda	deoxyribonucleic acid	1999-09-15	1.6	0.205	DM2	Atlas PDBsum SF
X-ray	365d	deoxyribonucleic acid	1999-01-20	1.6		NOD	Atlas PDBsum SF

Figure 14.3.12 The 40 PDB entries that include the 10 daunomycin-like ligands and access to their binding site details from MSDsite.

8. Click on the Get PDB entries URL; there are 40 PDB entries that include these ligands.

   By browsing through, one can also identify similarities in the biological function.

9. At the top right of the results screen, click on the Get PDB sites link to view details about the binding sites of these ten ligands in PDB entries. The view shown in Figure 14.3.12 (MSDsite search result page) appears. Follow a link for each PDB entry to visualize interactions of the ligands with the macromolecule, as well as further details and statistics regarding strength and distance of the interactions.

   There are more search options based on molecule graphs. The drop down menu for search operators next to the "Non stereo smile" label allow options for "exact structure" and "is substructure of" a particular structure. The exact structure search is instantaneous and will find all ligands that are stereoisomers of the input, using the "Non stereo smile" data field. If using the "Stereo smile" data field, which is just below, and the same "exact structure" search operator, one can search for particular stereoisomers but will need to input the correct stereo configuration in JME. The "Non stereo smile" "substructures of" search operator will return ligands that are included as subgraphs in the chemical graph provided as input.

Search using fingerprint similarity

10. Open the MSDchem search home page (*http://www.ebi.ac.uk/msd-srv/msdchem*; Fig. 14.3.1).

11. Type `DM1` directly into the "fingerprint" text field.

    For known ligands there is no need to use the JME editor in order to draw up their chemical structure. Giving a three-letter PDB code directly instead of a SMILE string is enough for MSDchem to automatically use its chemical structure as the search criteria.

    The Fingerprint search field uses fingerprints prepared using the existence of each one of 500 segments in the predefined library of the CACTVS system (Ihlenfeldt et al., 1992). This search option will give useful results mainly for big input molecules where the resulting hits will have almost the same segment groups (at least 99% common groups).

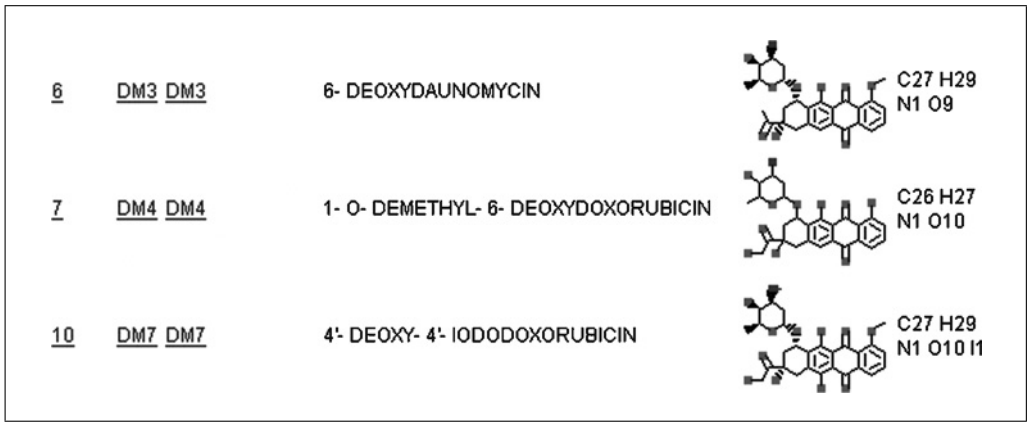

6 DM3 DM3 6- DEOXYDAUNOMYCIN C27 H29 N1 O9
7 DM4 DM4 1- O- DEMETHYL- 6- DEOXYDOXORUBICIN C26 H27 N1 O10
10 DM7 DM7 4'- DEOXY- 4'- IODODOXORUBICIN C27 H29 N1 O10 I1

Figure 14.3.13 Three more hits for DM1 daunomycin-like ligands revealed using the MSDchem fingerprint similarity searching.

12. Click on the Search button.

Giving the reduced DM1 as input will also return molecules that do not include the complete input structure but are still quite close in structure. For example, three more daunomycin/doxorubicin variants are found in this search (shown in Fig. 14.3.13) that were missed using the subgraph search. In this case the fingerprint similarity search proved quite useful, although one must take into to account that this option is in general more unpredictable than subgraph searching.

BASIC PROTOCOL 4

EXPORTING THE LIGAND DICTIONARY

Searching and downloading data for individual ligands is sufficient in most cases, but there are still times when it may be useful to have the complete ligand dictionary as a local resource for convenience or systematic use. The volume of data in the database is manageable and the MSDchem service offers several options for downloading it in various formats.

Necessary Resources

Hardware

Computer with Internet access

Software

An up-to-date Internet browser, such as Internet Explorer 3.0 or later (*http://www.microsoft.com/ie*); Netscape 4.75 or later (*http://browser.netscape.com*); Firefox 1.0 or later (*http://www.mozilla.org/firefox*)

A compression/decompression utility that can handle gzip-compressed tar files (WinZip for Windows, *http://www.winzip.com*; gzip, *http://www.gnu.org/software/gzip/gzip.html*, and tar, *http://www.gnu.org/software/tar*, for Linux and other Unix systems)

View the ligand index pages

1. Open the MSDchem search home page (*http://www.ebi.ac.uk/msd-srv/msdchem)*. Click on link to "ligand index and download" at lower left hand side to open an MSDchem ligand index page.

Ligand index pages provide direct links and data about ligands organized on a very simple layout. Ligands are listed numerically (0 to 9) and alphabetically (A to Z) according to the first character of their three-letter code. There are links to all of the index pages on the top of each index page for easy navigation. Each ligand is presented visually using a

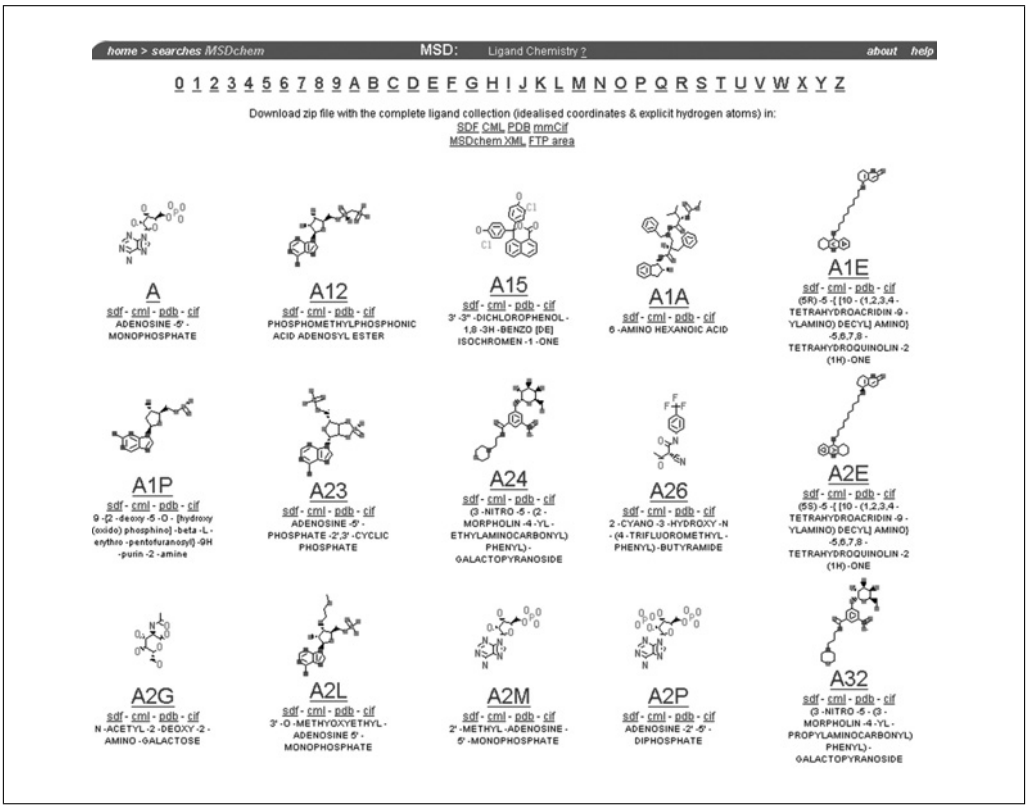

home > searches MSDchem
MSD: Ligand Chemistry ?
about help
0 1 2 3 4 5 6 7 8 9 A B C D E F G H I J K L M N O P Q R S T U V W X Y Z
Download zip file with the complete ligand collection (idealised coordinates & explicit hydrogen atoms) in:
SDF CML PDB mmCif
MSDchem XML FTP area
A
sdf - cml - pdb - cif
ADENOSINE -5' - MONOPHOSPHATE
A12
sdf - cml - pdb - cif
PHOSPHOMETHYLPHOSPHONIC ACID ADENOSYL ESTER
A15
sdf - cml - pdb - cif
3' -(3" -DICHLOROPHENOL - 1,8 -DIH -BENZO [DE] ISOCHROMEN -1 -ONE
A1A
sdf - cml - pdb - cif
6 -AMINO HEXANOIC ACID
A1E
sdf - cml - pdb - cif
(5R) -5 -[[10 - (1,2,3,4 - TETRAHYDROACRIDIN -9 - YLAMINO) DECYL] AMINO] -5,6,7,8 - TETRAHYDROQUINOLIN -2 (1H) -ONE
A1P
sdf - cml - pdb - cif
9 -[2 -deoxy -5 -O - [hydroxy (oxido) phosphino] -beta -L - erythro -pentofuranosyl] -9H -purin -2 -amine
A23
sdf - cml - pdb - cif
ADENOSINE -5' - PHOSPHATE -2',3' -CYCLIC PHOSPHATE
A24
sdf - cml - pdb - cif
(3 -NITRO -5 - (2 - MORPHOLIN -4 -YL - ETHYLAMINOCARBONYL) PHENYL) - GALACTOPYRANOSIDE
A26
sdf - cml - pdb - cif
2 -CYANO -3 -HYDROXY -N - (4 -TRIFLUOROMETHYL - PHENYL) -BUTYRAMIDE
A2E
sdf - cml - pdb - cif
(5S) -5 -[[10 - (1,2,3,4 - TETRAHYDROACRIDIN -9 - YLAMINO) DECYL] AMINO] -5,6,7,8 - TETRAHYDROQUINOLIN -2 (1H) -ONE
A2G
sdf - cml - pdb - cif
N -ACETYL -2 -DEOXY -2 - AMINO -GALACTOSE
A2L
sdf - cml - pdb - cif
3' -O -METHYDIXYETHYL - ADENOSINE 5' - MONOPHOSPHATE
A2M
sdf - cml - pdb - cif
2' -METHYL -ADENOSINE - 5' -MONOPHOSPHATE
A2P
sdf - cml - pdb - cif
ADENOSINE -2' -5' - DIPHOSPHATE
A32
sdf - cml - pdb - cif
(3 -NITRO -5 - (3 - MORPHOLIN -4 -YL - PROPYLAMINOCARBONYL) PHENYL) - GALACTOPYRANOSIDE

Figure 14.3.14 The MSDchem ligand index page for the letter A with a list of the 525 ligands that have a three-letter code starting with the character A. There are links to access and download data for each one of them as well as for the whole ligand collection.

small image of its chemical diagram, together with its common name and three-letter code for ligand identification. There are also links for the ligand details page and for chemical file format downloads. These pages can be useful for browsing ligands with interesting structure and for access to general purpose Web search engines that use components of ligand names.

2. Click on the letter A to obtain the view shown in Figure 14.3.14.

Download ligand files

3. Click on one of the four links at the top of each ligand page (Fig. 14.3.14) to download a gzip-compressed tar file for the complete ligand collection in a single compressed archive file. The file contains separate entries (~7000 as of this writing) for each ligand in one of the SDF/MDL, CML, PDB, or mmCif formats.

4. Alternatively, export an SDF/MDL, CML, PDB, or mmCif file for an individual ligand (e.g., DM1) by using the corresponding link in the ligand index page for letter D and clicking on the format link associated with DM1 (e.g., CML). The CML file for this entry will appear as shown in Figure 14.3.15.

Alternatively, the file may be saved in a temporary area and opened later using the appropriate program. Run the utility and export the files on a local directory.

Download a single file with summary data for all ligands

5. Click on the Export button on the MSDchem search home page (*http://www.ebi.ac.uk/msd-srv/msdchem*) to get a list of all ligands, together with their common names and SMILE strings, in a single XML file. Choose the output format from the Retrieve drop-down menu to get the list as a Perl or JavaScript data structure for easier programmatic processing.

Figure 14.3.15 The CML file for ligand DM1 as exported through the ligand index page for the letter D.

COMMENTARY

Background Information

The PDB ligand dictionary

The information available from the ligand dictionary is not part of the historical PDB archive. The PDB data bank files do not provide a clear chemical definition for ligands and amino/nucleic acids. Nevertheless, the PDB nomenclature based on three-letter codes and atom names clearly suggests that ligands with the same three-letter code should be the same chemical species and that atoms should superimpose within a stereochemical diagram. Of course, an explicit process to validate this rule has not always been in place, and the PDB archive has many errors propagated over the years. Furthermore, deriving the chemical identity of a ligand using a set of 3-D coordinates from a PDB entry is not a reliable operation, especially since there are inaccurate or unavailable experimental data in many cases.

The international body that manages the PDB is the Worldwide Protein Data Bank (wwPDB; Berman et al., 2005), with the mission of maintaining a single archive, freely and publicly available to the global community. wwPDB was founded by the Research Collaboratory for Structural Bioinformatics (RCSB PDB USA), the Macromolecular Structure Database group (MSD-EBI Europe) and the PDBj (Japan). All three organizations serve as deposition, data processing, and distribution sites for the PDB archive. Each site additionally provides its own view of the primary data with a variety of tools and resources for the global community.

There is an ongoing effort in wwPDB to address the problem of missing chemical definitions and to provide references and cleaned-up PDB data. At the time of curation of PDB entries, extensive work is done in carefully examining ligand chemistry issues and resolving them in cooperation with the depositors. The ligand dictionary (Westbrook et al., 2005), exchanged in the wwPDB (Chemical Component Information dictionary), that forms the basis of MSDchem, is a first step toward

achieving this goal. The wwPDB partners are making a systematic effort to finalize and resolve any remaining issues. This effort will ultimately be consolidated into a common dictionary. MSDchem incorporates the results of this effort, and at times it may provide data and corrections that are ahead of this common dictionary.

Ligand stereochemistry in MSDchem ligand dictionary

The MSDchem ligand dictionary includes explicit stereodescriptors using the absolute Chan-Ingold-Prelog notation as part of the definition of atoms and bonds in order to cope with the PDB implicit convention, i.e., different stereoisomers should be identified by different three-letter codes. Additionally, the MSDchem back-end system utilizes chemoinformatics programs and libraries like the CACTVS software package (Ihlenfeldt et al., 1992) and the CORINA Web service (Gasteiger et al., 1990) in order to enrich the ligand dictionary with important chemical information and to validate and clean-up the data collection.

The MSDchem view is that PDB coordinates and atom names are not fundamental properties of a ligand, which is defined as a complete, distinct stereoisomer of a chemical compound. Representative coordinates are used as speculative chemistry, and they require manual curation since a set of coordinates may be compatible with different isomers. On the other hand, there may be conflicts with the depositor's view of ligand chemistry introduced as errors in experimental data or in the refinement process. Therefore, the unique chemical identity of a ligand in MSDchem is based on its stereo SMILE string in the CACTVS canonical unique form, including automatic detection of aromaticity and tautomerism.

UNIT 1.9 describes in detail the PDB archive data and the RCSB PDB Web tools as well as Ligand Depot, the RCSB's Web search system for small molecule information. Ligand Depot, among others, provides access to various small molecule sites and resources, one of which is MSDchem.

Role of the MSDchem database

The MSDchem database provides the framework for the contribution of the Macromolecular Structure Database group in the wwPDB ligand curation and clean-up effort and for the correct processing of new PDB ligands and entries. It is based on the wwPDB chemical component information dictionary (Westbrook et al., 2005) and is an exchange resource; nevertheless, it also introduces several extensions like the use of explicit stereoconfiguration descriptors as part of the ligand identity.

Additionally, MSDchem has identified several cases where the same chemical species has been defined more than once using different three-letter codes. In these cases, one unique three-letter identifier has been selected and the remaining codes have been marked as obsolete in the MSDchem database in order to stop their use in new PDB entries. These obsolete ligands will be highlighted in all MSDchem result pages, with direct links to the entries that supersede them.

Topological variants

The MSDchem database also includes topological variants of small molecules (typically standard and modified amino/nucleic acids) that form polymeric chains. Amino acids, for example, usually have four entries for the same small molecule in the dictionary, one each for the free, N-terminal, O-terminal, and linking variants. This is apparent from the value of the "Extended code" column that is included in MSDchem results and ligand details pages. There is no predefined formula for the extended code values, and the format depends on the type of polymerization. However, the following standard naming conventions are used: <three-letter code>_LFOH for L-form of a free amino acid with OH group added; <three-letter code>_LL for L-form of a linking amino acid residue; <three-letter code>_LSN3 for L-form of a starting N-terminal (NH_3^+ group); <three-letter-code>_LEO2 for L-form of an ending O-terminal (O_2^- group).

The results from the protocols presented in the unit were retrieved from MSDchem release 28-2006_06_25. MSDchem is following the PDB weekly release cycle.

Critical Parameters and Troubleshooting

Even though the production of MSDchem requires systematic checking and corrections, it still contains several known errors and inaccuracies. These errors may be due to missing or problematic experimental data (e.g., incomplete single set of fully observed coordinates for some ligands or inconsistencies between the experimental coordinates and the chemical description), bugs or inaccuracies in chemical software packages (core dumps or error messages during

stereo-identification and idealized coordinate generation), or incomplete manual curation (e.g., valence inconsistencies or missing hydrogen atoms).

It is important to understand that construction of the MSDchem database and back-end system is an ongoing effort that will gradually improve the quality of the data collection and merge it into wwPDB. In addition, MSDchem provides a reference definition for a ligand that is not required to be consistent with data in the PDB archive. The task of validating and correcting the PDB data with ligand definitions is a separate effort that is only loosely associated with the quality of the data in MSDchem.

Finally, the MSDchem Web service has an additional restriction to avoid server and user overload. There is a maximum limit of 300 hits in any search. On exceeding this limit, the first 300 results are displayed along with a clear warning on the result page that the search has to be further refined.

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Finally, chemical software development projects like CACTVS and CORINA play a crucial role in providing supporting technology in the back-end of the MSDchem database.

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A consistent overview of the activities and policies of the MSD group at EBI and of the concepts of the MSD.

Westbrook et al., 2005. See above.

A description of the process of the wwPDB exchange, which is the basis of the MSDchem database.

Internet Resources

http://www.ebi.ac.uk/msd-srv/msdchem

The MSDchem search home page.

http://www.ebi.ac.uk/msd/index.html

Contains information about the MSD group and the MSD suite of tools and services.

http://www.ebi.ac.uk/msd-srv/msdlite

The MSDlite search system provides overview atlas pages for PDB entries, using the MSD database.

http://www.ebi.ac.uk/msd-srv/msdsite

The MSDsite Web service that provides details about ligand occurrences and binding sites of small molecules in PDB entries.

http://www.ebi.ac.uk/msd-srv/docs/dbdoc

Contains information about the MSDSD public search relational database and how to download and use it.

http://www.ebi.ac.uk/msd-srv/docs/moldoc/help.html

The molecule subgraph containment package used by the MSDchem search system.

http://deposit.pdb.org/public-component-erf.cif

The Chemical Component Information dictionary that is exchanged in wwPDB.

http://www2.chemie.uni-erlangen.de/software/cactvs

The CACTVS chemistry algorithm development environment, the main software package used by MSDchem database and Web service

http://www2.chemie.uni-erlangen.de/software/corina

The CORINA Web service for fast and efficient generation of high-quality 3-D molecular models used to generate idealized coordinates for ligands.

http://www.molinspiration.com/jme

The home page of the JME Molecular Editor Java applet used by MSDchem Web service.

http://jmol.sourceforge.net

The home page of the Jmol, free, open source 3-D molecule viewer used by MSDchem Web service.

http://www.mdli.com

Information about the definition of the popular MDL CTfile Formats.

http://www.acdlabs.com

The ACD-labs chemical software package used at the time of curation of new ligands.

http://users.unimi.it/~ddl/vega/index_noanim.htm

The VEGA Molecular modeling software package used in the back-end of the MSDchem database.

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In Silico Drug Exploration and Discovery Using DrugBank

DrugBank is a Web-based bioinformatics/cheminformatics resource that combines detailed drug data with comprehensive drug target information. It is primarily designed to facilitate computer-based drug and drug target discovery. Since it electronically catalogs almost all known drugs and drug targets, DrugBank is also used as a comprehensive online reference by pharmacists and pharmaceutical researchers. First released in January 2006, the DrugBank database is continuously being updated as new drugs are approved by the FDA or as new drug leads are identified (Wishart et al., 2006). DrugBank is fully searchable and supports extensive text, sequence, chemical structure, and relational database queries. Potential applications of DrugBank include computer-based drug target discovery, in silico drug design, drug docking or screening, drug metabolism prediction, drug interaction prediction, and general pharmaceutical education. In this unit, readers will be shown how to effectively navigate through and retrieve data from the DrugBank Web site (Basic Protocol 1), how to perform chemical structure similarity searches (Basic Protocol 2), and how to identify potential drug targets from newly sequenced pathogens (Basic Protocol 3).

Chemical structure similarity searching (Basic Protocol 2) is the chemical equivalent of searching a sequence database for sequence homologs or searching the Protein Data Bank (PDB; *UNIT 1.9*) for similar protein folds. It is particularly useful for organic chemists or natural product chemists who are interested in determining whether a newly synthesized compound or a newly identified natural product exhibits some similarity to a known drug. It is also useful for finding or comparing different compounds that are thought to be similar in structure. Drug target identification (Basic Protocol 3) essentially involves the identification of protein sequences from a newly sequenced pathogen that exhibit some similarity to the sequences of known drug targets. Presumably, if a novel virus or a newly identified pathogenic bacterium share some significant sequence similarity to a protein that is a known drug target from another organism, then the same (or similar) drugs may be used to treat this pathogen. Alternately, if they prove to be ineffective, these previously known drugs may serve as potential drug leads for developing more effective therapies. These protocols and their accompanying descriptions are intended to provide users with an appreciation of how cheminformatics (the study of chemical information) can be integrated into bioinformatics in a very practical and medically useful way.

NAVIGATING THE DrugBank WEB SITE

BASIC PROTOCOL 1

DrugBank can be accessed at: *http://redpoll.pharmacy.ualberta.ca/drugbank/*. It is a Web-accessible database that is structured to facilitate both casual browsing and directed searching. DrugBank is compatible with most modern Web browsers (equipped with a Java interpreter) and is designed to be navigated using standard hyperlinked menus or hyperlinked text. The appearance of the database and its functionality should be the same, regardless of the user's browser or operating system. As with any online tool, DrugBank has a home page with a hyperlinked menu bar located at the top of the page. This menu bar allows users to easily move back and forth between specific display or query pages. Directed queries or text and sequence searches are typically done by typing or pasting text into standard text boxes and the query function is activated by pressing a "Search" or "Submit" button. The home page also provides a brief overview of the database and some of its features. This protocol describes in detail how users can find, view, interpret, and retrieve data from the DrugBank Web site.

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Necessary Resources

Hardware

Computer with Internet access

Software

An up-to-date Internet browser, such as Internet Explorer (*http://www.microsoft.com/ie*); Netscape (*http://browser.netscape.com*); Firefox (*http://www.mozilla.org/firefox*); or Safari (*http://www.apple.com/safari*). The Web browser must be capable of handling Java Applets (i.e., equipped with a Java interpreter) and capable of opening and viewing PDF files.

Files

None

Standard DrugCard overview

1. Go to the DrugBank Web site at *http://redpoll.pharmacy.ualberta.ca/drugbank/*.

   The DrugBank home page (Fig. 14.4.1) has a blue menu bar located near the top of the page with eight clickable titles: ***Home, Browse, PharmaBrowse, ChemQuery, TextQuery, SeqSearch, Data Extractor****, and* ***Download****. This menu bar, which appears near the top of every DrugBank Web page, allows users to easily navigate to the different browsing and search utilities in the database. Below the menu bar is a text box with the phrase* ***Search DrugBank for****. This text search utility, which is the most commonly used search feature in DrugBank, is also displayed near the top of nearly every DrugBank Web page. Below this is a brief description of DrugBank and some of the features contained in the database. Users may refer to this page for more information on how to use DrugBank or to get the latest information on what is contained in it.*

DrugBank Homepage - Netscape
File Edit View Go Bookmarks Tools Window Help
http://redpoll.pharmacy.ualberta.ca/drugbank/index.html
DrugBank
-Home- Browse PharmaBrowse ChemQuery TextQuery SeqSearch Data Extractor Download
Search DrugBank for: tricyclic
Search
The DrugBank database is a unique bioinformatics and cheminformatics resource that combines detailed drug (i.e. chemical, pharmacological and pharmaceutical) data with comprehensive drug target (i.e. sequence, structure, and pathway) information. The database contains nearly 4300 drug entries including >1,000 FDA-approved small molecule drugs, 113 FDA-approved biotech (protein/peptide) drugs, 62 nutraceuticals and >3,000 experimental drugs. Additionally, more than 6,000 protein (i.e. drug target) sequences are linked to these drug entries. Each DrugCard entry contains more than 80 data fields with half of the information being devoted to drug/chemical data and the other half devoted to drug target or protein data.
Please cite: Wishart DS et al., DrugBank: a comprehensive resource for in silico drug discovery and exploration. Nucleic Acids Res. 2006 1;34
Users may query DrugBank in any number of ways. The simple text query (above) supports general text queries of the entire textual component of the database. Clicking on the Browse button (on the DrugBank navigation panel above) generates a tabular synopsis of DrugBank's content. This browse view allows users to casually scroll through the database or re-sort its contents. Clicking on a given DrugCard button brings up the full data content for the corresponding drug. A complete explanation of all the DrugCard fields and sources is given here. The PharmaBrowse button allows users to browse through drugs as grouped by their indication. This is particularly useful for pharmacists and physicians, but also for pharmaceutical researchers looking for potential drug leads. The ChemQuery button allows users to
start

Figure 14.4.1 Screen shot of the DrugBank home page. At the top of the page is a menu bar (blue on screen) containing eight menu choices (in white). Users can navigate through DrugBank using this menu bar. Details of how to use, contact, and reference DrugBank are given in the central text window.

2. Type `tricyclic` into the Search DrugBank for text box and then press the Search button. A three-column table should appear within a few seconds, containing a list of almost all known tricyclic antidepressant drugs, as well as other tricyclic molecules (Fig. 14.4.2). The first column displays the DrugBank accession number (which is hyperlinked); the second column displays the drug's generic (or common) name, while the third column displays the chemical formula.

   The text search is a non-case-sensitive utility that supports searches of complete words, numbers, multiple words, phrases, and partial words (be careful, numeric searches can take a while). Users could enter `tryc` *or* `tricyclic anti`*, for example, and similar results would be returned (23 to 30 hits). Not all of the drugs listed are tricyclic antidepressants, as they only contain the quoted word or word segments somewhere in their data files. It is important to mention the fact that DrugBank's text search utility is not restricted to drugs or drug names—it is able to search through most any text in the database, including gene and protein names that are known drug targets. Note that the text search tool does not search through sequence text. The search engine uses a rapid index-based query tool called GLIMPSE (Manber and Bigot, 1997). Hits are ordered according to the DrugBank accession numbers, with FDA-approved drugs given first (marked by an* `APRD` *prefix), followed by experimental or preclinical drugs (marked by an* `EXPT` *prefix). Biotech drugs are given the* `BIOD` *prefix.*

3. Click on the hyperlinked accession number for desipramine (`APRD00022`). A new window should be launched containing the "DrugCard" for desipramine (Fig. 14.4.3).

   All of the drugs contained in DrugBank have their information listed in individual DrugCards—in analogy to the very successful GeneCards concept (Rebhan et al., 1998). Each DrugCard entry contains >80 data fields, with half of the information being devoted to drug/chemical data and the other half devoted to drug target or protein data (see Table 14.4.1). More specifically, the DrugCard information is ordered as follows: (1) drug nomenclature; (2) physical properties; (3) structural data; (4) pharmacological

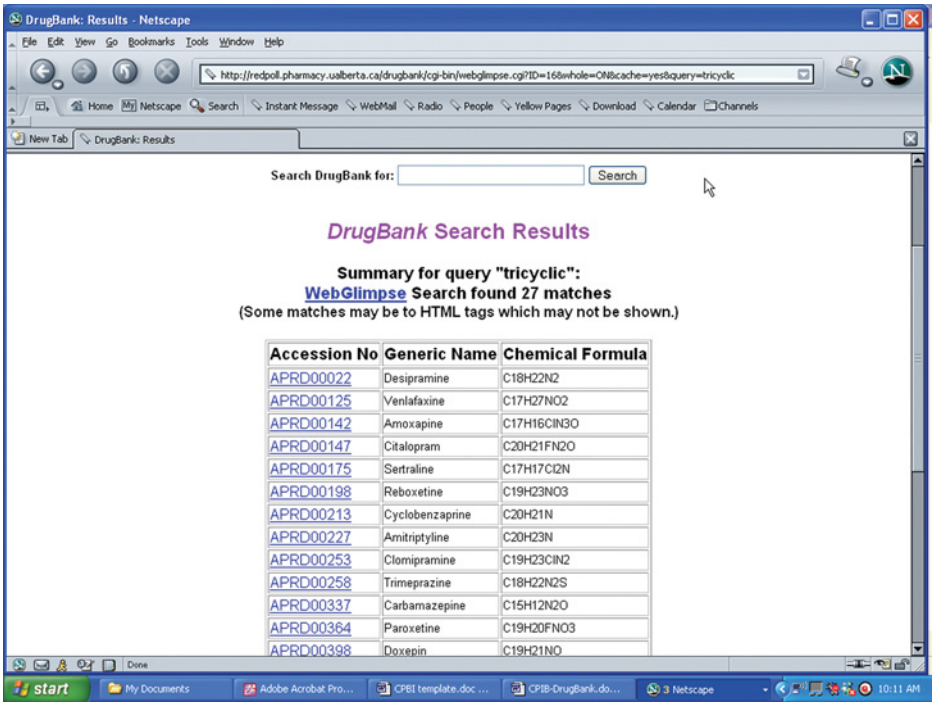
DrugBank: Results - Netscape
Search DrugBank for:
Search
DrugBank Search Results
Summary for query "tricyclic":
WebGlimpse Search found 27 matches
(Some matches may be to HTML tags which may not be shown.)
Accession No | Generic Name | Chemical Formula
APRD00022 | Desipramine | C18H22N2
APRD00125 | Venlafaxine | C17H27NO2
APRD00142 | Amoxapine | C17H16ClN3O
APRD00147 | Citalopram | C20H21FN2O
APRD00175 | Sertraline | C17H17Cl2N
APRD00198 | Reboxetine | C19H23NO3
APRD00213 | Cyclobenzaprine | C20H21N
APRD00227 | Amitriptyline | C20H23N
APRD00253 | Clomipramine | C19H23ClN2
APRD00258 | Trimeprazine | C18H22N2S
APRD00337 | Carbamazepine | C15H12N2O
APRD00364 | Paroxetine | C19H20FNO3
APRD00398 | Doxepin | C19H21NO

Figure 14.4.2 A screen shot of a Search DrugBank for query output for the word "tricyclic." The drug names on the left side of the table are hyperlinked.

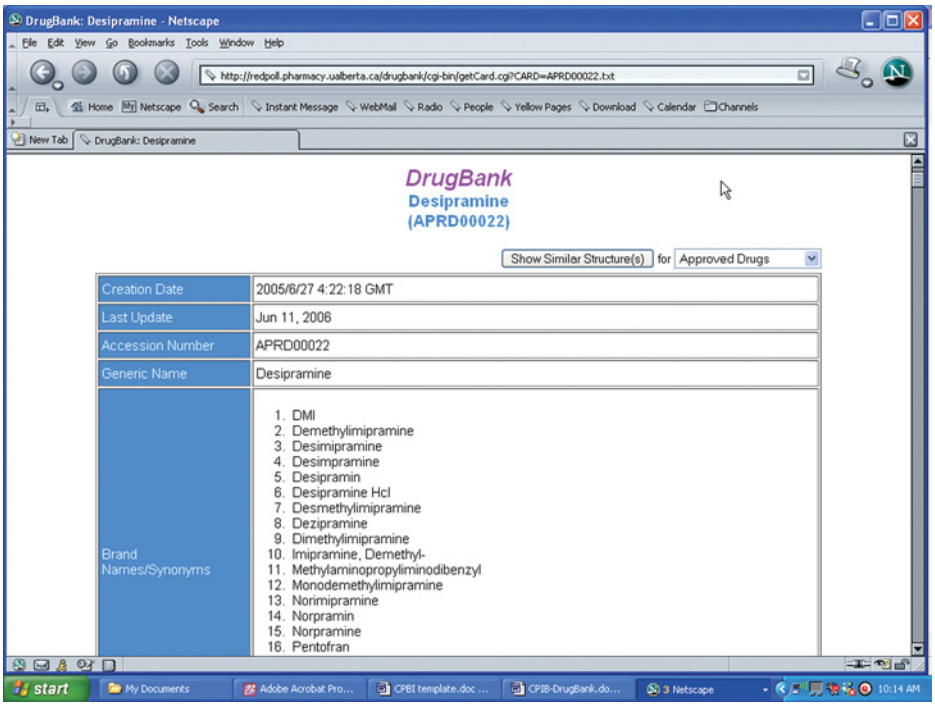

Figure 14.4.3 A screen shot of the DrugCard for desipramine, a tricyclic antidepressant.

data; (5) drug target or target protein data; and (6) genetic and/or SNP data for the target protein. If a drug has more than one biomolecular target, the protein and genetic data fields are repeated for each protein target. In addition to providing comprehensive numeric, sequence, and textual data, each DrugCard also contains hyperlinks to other databases, abstracts, digital images, and interactive applets for viewing molecular structures.

4. To get better acquainted with the type of information contained in a typical DrugCard, use the scroll bar on the right side of the browser's window to scroll down the desipramine DrugCard page. Field names or titles are given on the left side of the table, while drug-specific descriptors are given on the right. The top portion of each DrugCard is devoted to providing detailed information about the names, synonyms, chemical structure, and general information for that drug. Some of the fields contain hyperlinks (marked in light blue text). Click on the KEGG Compound ID hyperlink (see *UNIT 1.12*). This will launch a new window to the KEGG Web page for desipramine. Close this window, then click on the PubChem ID (Substance or Compound) hyperlink. This will launch the PubChem page for desipramine. After viewing the PubChem site, close its window. The desipramine DrugCard should still be visible.

   The chemical and biological information contained in DrugBank is assembled from more than a dozen textbooks, several hundred journal articles, nearly 30 different electronic databases, and at least 20 in-house or Web-based programs were individually searched, accessed, compared, written, or run over the course of four years. The original team of DrugBank archivists and annotators included two accredited pharmacists, a physician, and three bioinformaticians with dual training in computing science and molecular biology/chemistry. Manual updates of the database are continuing, although many annotation fields are now being automatically updated and added using customized text-mining programs.

5. To view an editable 2-D image of desipramine, scroll down to the data field called **MOL File Image** and click on the hyperlinked button called **View 2D Structure**.

Table 14.4.1 Summary of the Data Fields or Data Types Found in Each DrugCard[a]

Drug or compound information	Drug target or receptor information
Generic name	Target name
Brand name(s)/synonyms	Target synonyms
IUPAC name	Target protein sequence
Chemical structure/sequence	Target no. of residues
Chemical formula	Target molecular weight
PubChem/KEGG/ChEBI Links	Target pI
SwissProt/GenBank Links	Target gene ontology
FDA/MSDS/RxList Links	Target general function
Molecular weight	Target specific function
Melting point	Target pathways
Water solubility	Target reactions
pKa or pI	Target Pfam domains
LogP or hydrophobicity	Target signal sequences
NMR/MS spectra	Target transmembrane regions
MOL/SDF/PDF text files	Target essentiality
MOL/PDB image files	Target GenBank Protein ID
SMILES string	Target SwissProt ID
Indication	Target PDB ID
Pharmacology	Target cellular location
Mechanism of action	Target DNA sequence
Biotransformation/absorption	Target chromosome location
Patient/physician information	Target locus
Metabolizing enzymes	Target SNPs/mutations

[a]A more complete listing is provided on the DrugBank home page.

This launches ACD's ChemSketch Java applet. After a few seconds, an image of desipramine should appear in the applet window (Fig. 14.4.4). If it does not, this likely indicates that the browser being used lacks the Java Virtual Machine and needs upgrading. The ChemSketch applet used to display this image allows the user to interactively alter, view, rotate, or zoom into the structure and to cut/paste the image (or altered image) into other files.

In addition to structural images of the drug, MOL and SDF text files are also available. MOL and SDF files are standard formats used by chemists to exchange and render 2-D chemical structure information. These files can be downloaded by the user and used to display or re-render the structures using higher-end commercial chemistry software packages like ChemDraw, ChemSketch, or IsisDraw.

6. A 3-D structure of desipramine can also be viewed by clicking on the hyperlinked button **View 3D Structure** contained in the PDB File Calculated Image field. This launches the WebMol interactive 3-D viewing applet (Fig. 14.4.5). The Calculated 3-D structure is generated via CORINA (Sadowski and Gasteiger, 1993). If an experimental set of 3-D coordinates (in PDB) is available, these may be viewed in a similar manner. 3-D coordinate files can also be downloaded and the structure can be minimized or a molecular dynamics run may be performed (using third-party software) to generate multiple conformers of the drug. These structures may be used

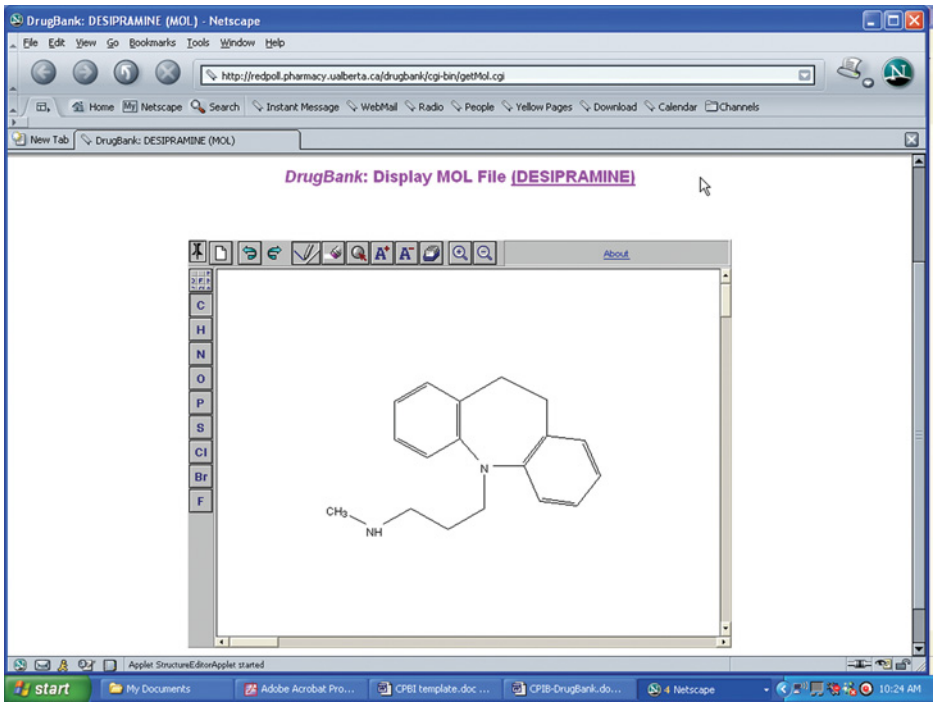

Figure 14.4.4 A 2-D image of the structure of desipramine as displayed using the ChemSketch Java applet. The image may be manipulated for different display purposes.

for ligand docking experiments with such tools as GLIDE (*UNIT 8.12*) or FLEXX (Kramer et al., 1997; Halgren et al., 2004).

CORINA is a rule-based structure generation program that has been shown to generate very accurate 3-D structures from 2-D chemical sketches. These calculated structures typically differ from the experimentally determined structures by no more than 0.4 Å. The same WebMol applet that is used to display small molecule drugs in DrugBank can also be used to display protein structures of either the drug target or of certain biotech drugs (e.g., BIOD00017). WebMol is a fast, flexible viewing tool that allows users to rotate, zoom, color, stereoview, measure, label, and selectively display different parts of a molecule. More information about WebMol and how to use it can be found at http://www.cmpharm.ucsf.edu/~walther/webmol.html.

7. Continue to scroll down the desipramine DrugCard. Numerous fields containing detailed pharmaceutical, pharmacological, and clinical data should be seen (e.g., **Drug Category, Indication, Pharmacology, Absorption, Toxicity, Half Life, Interactions**), as well as hyperlinks to different online **Drug References** (RxList and Drugs.com). Click on these to see what additional clinical information is available on desipramine. Remember to close the window once the information has been viewed. Scrolling further down on the desipramine DrugCard a table separator labeled **Drug Target 1** should be seen. This marks the beginning of the biological data on the genes and proteins that this drug is known to target. For instance, desipramine is known to bind to the M1 muscarinic acetylcholine receptor. As seen in this section, a considerable amount of detailed information is provided about this protein. This information can be particularly useful if one is planning on purifying, cloning, or working with the protein for any prospective drug assays.

DrugBank is particularly notable for the amount of biological information it provides about known drug targets. Many of its annotations are obtained through direct database comparisons to SwissProt and UniProt (Bairoch et al., 2005) or "guilt-by-association"

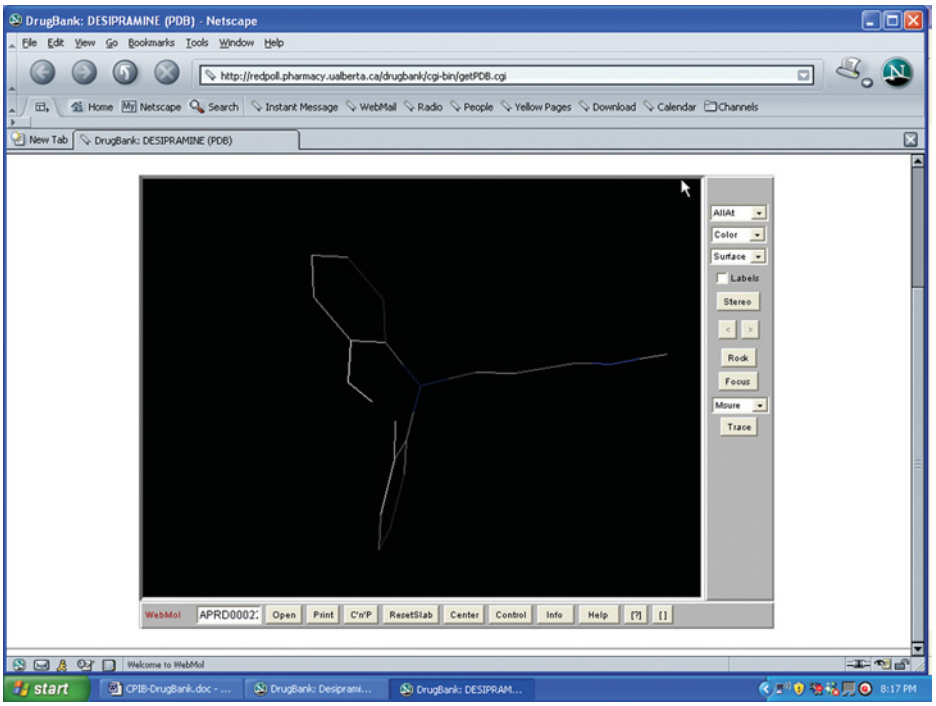

Figure 14.4.5 An image of the 3-D structure of desipramine as displayed using the WebMol Java applet. Users may manipulate the image for better viewing or further analysis.

determinations through BLAST searches. However, other annotations are obtained independently using a variety of in-house predictive programs, including protein family analysis with Pfam (Bateman et al., 2004), sequence motif analysis with PROSITE (Hulo et al., 2004), signal peptide and transmembrane domain prediction with TM-HMM (Krogh et al., 2001), and secondary structure prediction with PSIPRED and PROTEUS (McGuffin et al., 2000; Montgomerie et al., 2006). If the sequence has >35% sequence identity to a sequence represented in the PDB database, a homology model is generated using a program called HOMODELLER and subsequent structural analyses are performed using VADAR (Willard et al., 2003). Several additional annotations, such as molecular weight, amino acid content, and isoelectric point are calculated directly from the amino acid sequence using well-known algorithms and formulae.

8. Near the bottom of the Drug Target 1 section of the desipramine DrugCard will be a detailed list containing information about the Single Nucleotide Polymorphisms or **Drug Target 1 SNPs** associated with the M1 muscarinic acetylcholine receptor gene (Fig. 14.4.6). This multicolumn table provides a hyperlink to the refSNP ID, the type of SNP (synonymous or nonsynonymous), level of validation, base changes, position of the SNP, the consequent amino acid changes (if applicable), the amino acid position (if applicable), and the allele frequencies in different populations. SNP information is particularly important for understanding the origins for certain diseases, the propensity for individuals to get certain diseases and, most particularly, the cause of adverse drug reactions (ADRs).

ADRs are unexpected, drug induced problems that adversely affect the health of a patient. Sometimes they are called drug "allergies." Some individuals and some ethnic groups are known to have strong reactions to certain drugs or to require significantly different dosing regimens than other individuals or groups. Many of these differences are likely due to SNPs in either the drug target or a drug metabolizing enzyme. Recently, several drugs (aripiprazole, atomoxetin, and celecoxib) have received FDA approval for only targeted segments of the population having the appropriate SNP genotype. Adverse drug

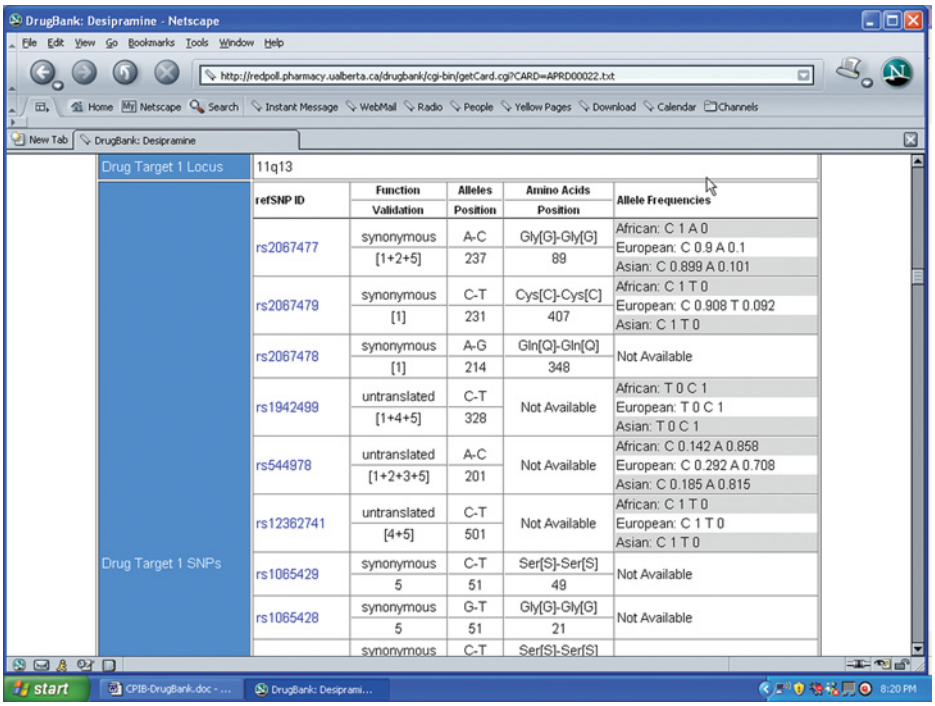
DrugBank: Desipramine - Netscape
Drug Target 1 Locus
11q13
Drug Target 1 SNPs
refSNP ID
Function Validation
Alleles Position
Amino Acids Position
Allele Frequencies
rs2067477 synonymous [1+2+5] A-C 237 Gly[G]-Gly[G] 89 African: C 1 A 0 European: C 0.9 A 0.1 Asian: C 0.899 A 0.101
rs2067479 synonymous [1] C-T 231 Cys[C]-Cys[C] 407 African: C 1 T 0 European: C 0.908 T 0.092 Asian: C 1 T 0
rs2067478 synonymous [1] A-G 214 Gln[Q]-Gln[Q] 348 Not Available
rs1942499 untranslated [1+4+5] C-T 328 Not Available African: T 0 C 1 European: T 0 C 1 Asian: T 0 C 1
rs544978 untranslated [1+2+3+5] A-C 201 Not Available African: C 0.142 A 0.858 European: C 0.292 A 0.708 Asian: C 0.185 A 0.815
rs12362741 untranslated [4+5] C-T 501 Not Available African: C 1 T 0 European: C 1 T 0 Asian: C 1 T 0
rs1065429 synonymous 5 C-T 51 Ser[S]-Ser[S] 49 Not Available
rs1065428 synonymous 5 G-T 51 Gly[G]-Gly[G] 21 Not Available

Figure 14.4.6 Details of the SNP (single nucleotide polymorphism) drug target information contained in the DrugCard for desipramine.

reactions are not a trivial problem. They lead to an average of two million hospitalizations, 100,000 deaths and thousands of malpractice suits per year in the United States alone. Furthermore, approved-drug withdrawals due to adverse drug reactions can cost pharmaceutical companies billions of dollars (recall Vioxx and Bextra).

9. Scroll down further through the desipramine DrugCard. The DrugCard will contain 6 more drug targets for desipramine including the Sodium-dependent norepinephrine re-uptake pump, the beta-2 adrenergic receptor, the M2 muscarinic acetylcholine receptor, the sodium-dependent serotonin re-uptake pump, the histamine H1 receptor, and the beta-1 adrenergic receptor. A quick check will reveal that similarly detailed biochemical and genetic data is available for each of these targets.

Using the DrugBank Browser and PharmaBrowse tools

The following part of the protocol involves learning how to use the navigation and search tools listed in the DrugBank menu bar.

10. Return to the DrugBank home page (press the back button on the browser or re-enter the DrugBank URL). Click on the Browse hyperlink located in the left side of the DrugBank menu bar. This launches the DrugBank Browser (Fig. 14.4.7).

The DrugBank Browser consists of a multi-page summary table listing all the drugs in DrugBank. Each browser page contains a formatted list of ~20 drugs that includes the DrugBank accession code, the generic (or common) drug name, the molecular formula (and weight), a thumbnail image of the drug structure, the CAS (Chemical Abstract Service) number, the therapeutic indication (the disease or condition it is used to treat), and the drug class—for each drug. Using the DrugBank Browser users may navigate through DrugBank in a slightly different way than through the text search tool. In particular, the DrugBank Browser allows users to select or sort drugs on the basis of drug class, accession code, or molecular weight. Clicking on the DrugCard button found in the leftmost column of any given DrugBank browser table opens the corresponding DrugCard.

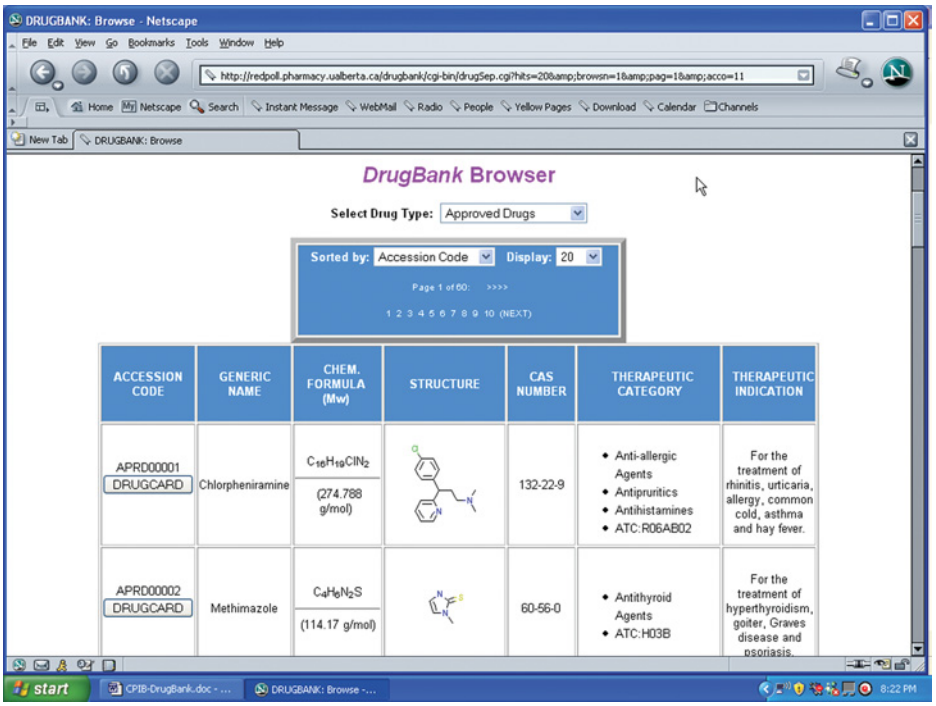

Figure 14.4.7 A screen shot of the DrugBank Browser. Note the tabular format and the sorting/display tools at the top of the Browser page.

11. At the top of the DrugBank Browser users may use the pull-down menu tab called **Select Drug Type** to select the type of drug class they wish to view. Users may choose between FDA-approved Drugs, Experimental, Nutraceutical, Biotech, Small Molecule Drugs (approved and experimental), and All Compounds (Experimental + Approved). The default class is FDA-approved Drugs. For this step in the protocol, select the Biotech drug class from the pull-down menu. The Browser page should automatically change to the screen shown in Figure 14.4.8.

    DrugBank contains ~1100 FDA-approved small molecule drugs, 120 FDA-approved biotech (protein/peptide) drugs, 65 nutraceuticals or micronutrients such as vitamins and metabolites, and 3200 experimental drugs, including unapproved drugs, de-listed drugs, illicit drugs, enzyme inhibitors, and potential toxins (Wishart et al., 2006). Generally much less pharmacological and biophysical data is available for the experimental drugs than for the approved drugs. Users may note that the Biotech drugs contain "generic" structures for their thumbnail images rather than detailed chemical structures as seen for the small molecule drugs. This is because most Biotech drug molecules are simply too large and too complex to show anything meaningful in a thumbnail sketch.

12. Below the Select Drug Type pull-down tab is a rectangular blue box that serves as the Browser's sorting and reformatting interface. Within this box is a **Sort By** pull-down tab. Users may sort any given DrugBank summary table by the DrugBank accession code, generic name, molecular weight, CAS number, therapeutic category, or therapeutic indication. Using the **Display** pull-down tab, users may also reformat the table to display 20, 50, 100, or 200 drugs per page. A repagination selector at the bottom of the box allows users to navigate from one page to another or to jump from one page to another quickly simply by clicking the hyperlinked page numbers or arrows. Sort the table by Molecular Weight and use the Display tab to show 100 drugs per page. The results should look like what is shown in Figure 14.4.9.

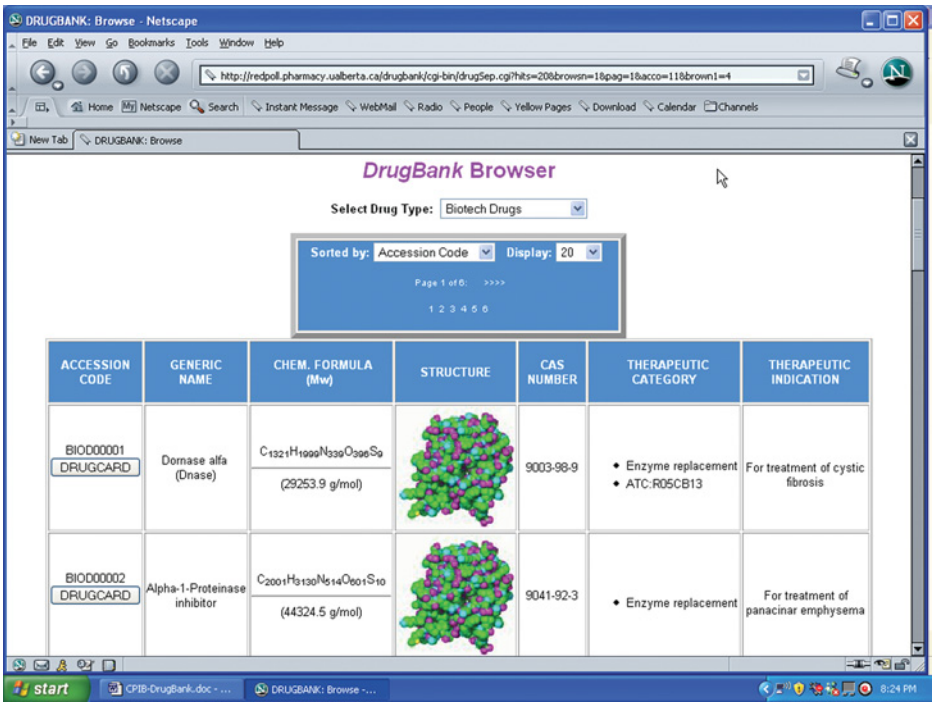

Figure 14.4.8 A screen shot of the DrugBank Browser set to display biotech (i.e., protein) drugs.

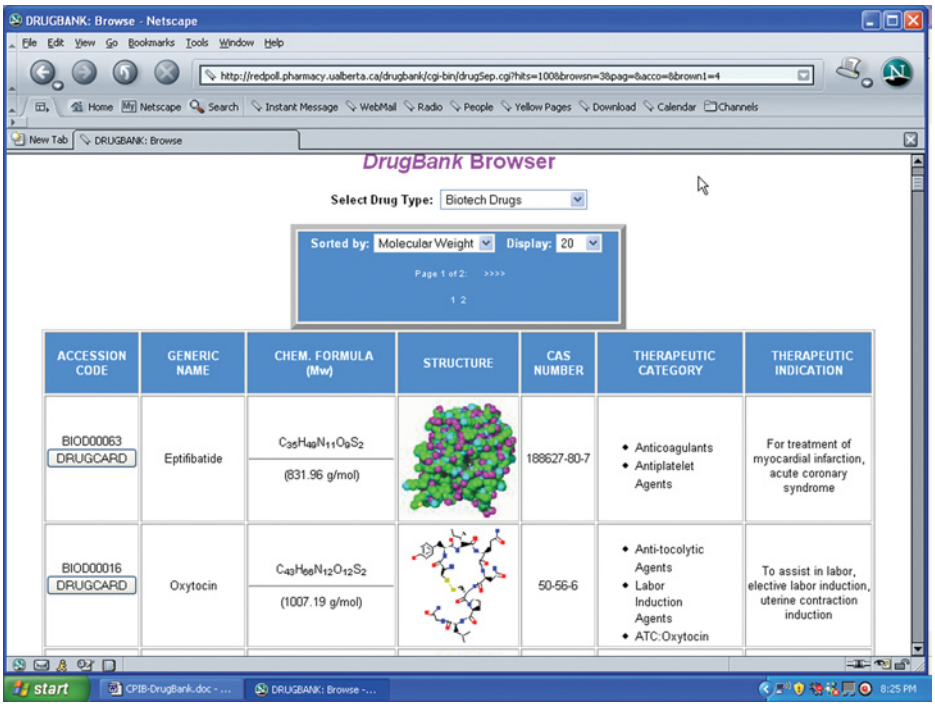

Figure 14.4.9 A screen shot of the DrugBank Browser set to display biotech drugs sorted by molecule weight (from smallest to largest).

The selecting, sorting, reformatting, and repagination features of the DrugBank Browser are particularly useful for surveying particular classes of drugs and getting a global picture of certain drug characteristics. Users may notice certain trends or patterns in the structure or therapeutic indications of selected drugs. They may also easily identify the largest or smallest drugs or quickly count up the total number of drugs belonging to a particular class or therapeutic indication. In the example shown here, it can be seen that the smallest biotech drug is eptifibatide, with a molecular weight of 831.96 Da. The DrugBank Browser is primarily intended to facilitate undirected exploration of the database and it allows users with no experience or little knowledge in the area of drugs or drug chemistry to easily access the material in the DrugBank database.

13. Go to the DrugBank menu at the top of the page and select **PharmaBrowse**. The following window should appear (Fig. 14.4.10). Scroll down to the list until the table heading called **NERVOUS SYSTEM** can be seen**.** Click on the hyperlink below this title called **PSYCHOANALEPTICS**. This should jump the page down to a list of FDA-approved psychoanaleptic drugs (central nervous system stimulants that reverse depression). Note that desimaprine is listed at the top of the page, along with 37 other drugs.

This particular browsing tool, which is also called the DrugBank category browser, provides navigation hyperlinks to 14 major drug categories which are then divided into more than 70 drug classes. Each drug class contains the generic names of all the FDA-approved drugs associated with that drug class. Each drug name is then linked to its respective DrugCard. Within PharmaBrowse, users may select Approved Drugs, Biotech Drugs, or Nutraceuticals by clicking on the hyperlinks at the top of the table. The PharmaBrowse browser is distinct from the regular DrugBank browser, as it was designed to address the specific needs of pharmacists, physicians, and medicinal chemists. These individuals tend to think of drugs in clusters of indications or drug classes. This allows them to identify alternate drug therapies (clinicians) or to look for common structural themes (medicinal chemists). On the other hand, biochemists and molecular biologists tend to think of drugs as stand-alone substrates, templates, or ligands. This view is more compatible with the conventional DrugBank Browser presented in steps 11 and 12.

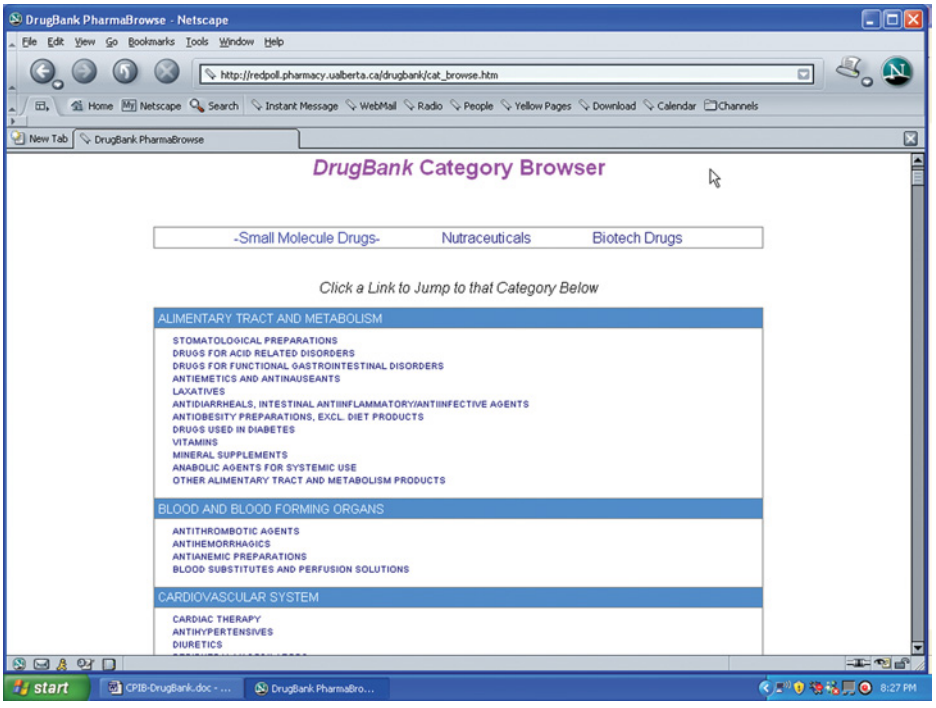

Figure 14.4.10 A screen shot of the PharmaBrowse browsing/navigation page. Note the hyperlinked list of drug categories and general indications.

Using the Text Query search option

14. Go to the DrugBank menu at the top of the page again and click on the **Text Query** hyperlink. The window shown in Figure 14.4.11 should appear. The Text Query tool allows users to perform more complex text queries in DrugBank than would be possible using the simple text search system (Search DrugBank for) found on the home page.

    With Text Query, users may perform case-sensitive queries, queries with multiple misspellings, partial or complete word matches (partial word matches are the default), along with phrases containing Boolean (AND, OR, NOT, etc.) operators. Upper limits on the number of files and number of word matches within a file (200 is the default) are also adjustable.

15. In the text box, type `tricyclic AND antidepressant` and press the Submit button. Within a few seconds, a hyperlinked list of the 25 known tricyclic antidepressants, including desipramine, should appear. Note that this query is obviously more specific than the simple text query done in Step 4.

    Like the general Text Query tool, the Text Search tool supports searches of complete words, numbers, multiple words, phrases, and partial words among most data fields (except sequence text) in DrugBank. Text Search uses the full version of GLIMPSE (Manber and Bigot, 1997) to allow very rapid search and retrieval of text data.

Using the Data Extractor search option

16. Go to the DrugBank menu at the top of the page again and click on the **Data Extractor** hyperlink. The following window, as seen in Figure 14.4.12, should appear. The Data Extractor was developed to allow users to perform more complex searches than what is possible through DrugBank's Search DrugBank for, Text Search, Browse, or PharmaBrowse utilities.

DrugBank Text Query - Netscape
File Edit View Go Bookmarks Tools Window Help
http://redpoll.pharmacy.ualberta.ca/drugbank/drugTextSearch.htm
Home Netscape Search Instant Message WebMail Radio People Yellow Pages Download Calendar Channels
New Tab DrugBank Text Query
DrugBank
Home Browse PharmaBrowse ChemQuery -Text Query- SeqSearch Data Extractor Download
This project is supported by Genome Alberta & Genome Canada, a not-for-profit organization that is leading Canada's national genomics strategy with $600 million in funding from the federal government.
DrugBank Text Search
DrugBank Text Search support partial or whole word search, and "And", "Or", "Not" operation.
Search for:
Submit
Reset
Case sensitive
Partial match
Use filters
Misspellings allowed: 0
Maximum number of files returned: 200
Maximum number of matches per file returned: 200
Glimpse & WebGlimpse (Copyright © 1996, University of Arizona)
DrugBank Database (Copyright © 2001, University of Alberta)
start
CPIB-DrugBank.doc - ...
DrugBank Text Query...
8:29 PM

Figure 14.4.11 A screen shot of the Text Query window. This permits more complex text queries than what is available through the default Search tool.

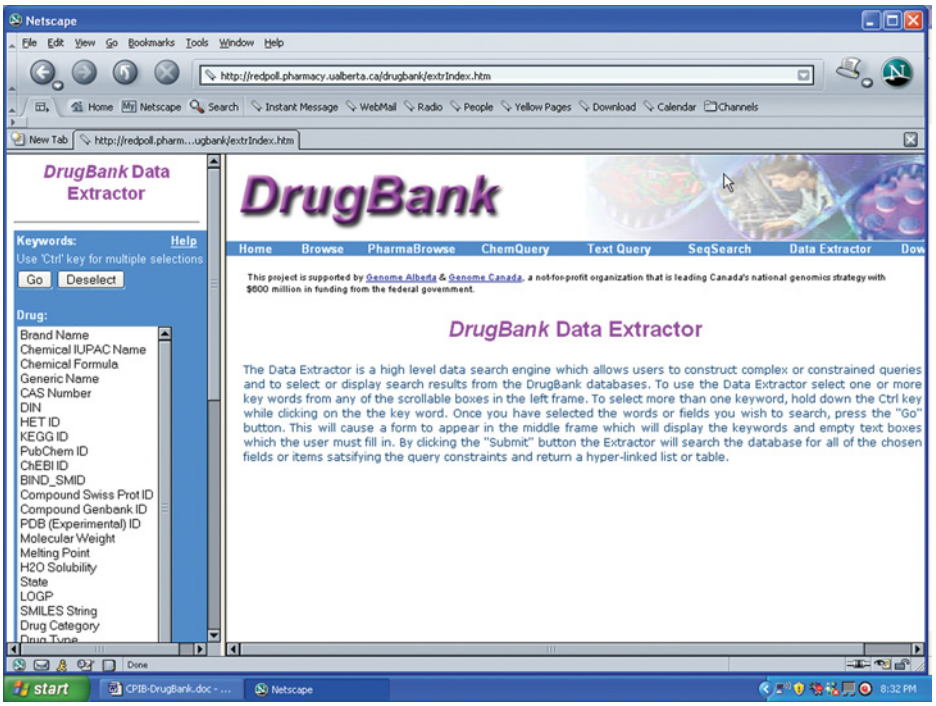
Netscape
File Edit View Go Bookmarks Tools Window Help
http://redpoll.pharmacy.ualberta.ca/drugbank/extrIndex.htm
DrugBank Data Extractor
Keywords: Help
Use 'Ctrl' key for multiple selections
Go Deselect
Drug:
Brand Name
Chemical IUPAC Name
Chemical Formula
Generic Name
CAS Number
DIN
HET ID
KEGG ID
PubChem ID
ChEBI ID
BIND_SMID
Compound Swiss Prot ID
Compound Genbank ID
PDB (Experimental) ID
Molecular Weight
Melting Point
H2O Solubility
State
LOGP
SMILES String
Drug Category
DrugBank
Home Browse PharmaBrowse ChemQuery Text Query SeqSearch Data Extractor
DrugBank Data Extractor
The Data Extractor is a high level data search engine which allows users to construct complex or constrained queries and to select or display search results from the DrugBank databases. To use the Data Extractor select one or more key words from any of the scrollable boxes in the left frame. To select more than one keyword, hold down the Ctrl key while clicking on the the key word. Once you have selected the words or fields you wish to search, press the "Go" button. This will cause a form to appear in the middle frame which will display the keywords and empty text boxes which the user must fill in. By clicking the "Submit" button the Extractor will search the database for all of the chosen fields or items satsifying the query constraints and return a hyper-linked list or table.
start
8:32 PM

Figure 14.4.12 A screen shot of the Data Extractor window. The Data Extractor supports advanced SQL-like searches through many different data fields.

DrugBank's Data Extractor employs a simple relational database system that allows users to select one or more data fields and to search for ranges, occurrences or partial occurrences of words, strings, or numbers. Using a few mouse clicks it is relatively simple to construct very complex queries ("find all drugs less than 300 Da with LogPs between 3.4 and 4.2 that are antidepressants") or to build a series of highly customized tables. The output from these Data Extractor queries is provided as an HTML format with hyperlinks to all associated DrugCards.

17. The central window frame provides a brief description of how to use the Data Extractor. On the left side of the Data Extractor is a smaller window frame (the selector window frame) with two scrollable/selectable lists, one titled **Drug** and the other titled **Drug Targets**. At the top and bottom of these lists are two buttons, **Go** and **Deselect**. Scroll down the first list and use the mouse to click on the word **Molecular Weight**. Molecular Weight should now be highlighted. Scroll down further and, *while holding down the "Ctrl" key*, click on **LOGP** and then **Drug Category**. In total, three query words should be highlighted. These query words represent the data fields that will be used to fine-tune the final query (i.e., finding a drug with specified features pertaining to molecular weight, LogP, and drug category).

This list highlighting process is actually being used to build the structured query language (SQL) query that is used to search the DrugBank database. Rather than having users learn SQL, this graphical user interface allows users to construct complex queries by simply selecting different data fields from a scrollable list. The scrollable list is generated using a JavaScript tool and so, unfortunately, it is not consistently formatted from browser to browser. If the list of Drug and Drug Target terms is cut off, move the mouse over the right border region of the smaller window. A double-sided horizontal arrow or similar image should appear. Click on the mouse and drag the window border so that the full list of terms and scroll bars is viewable.

18. Now, go to the top of the selector window frame and click on the **Go** button. A central blue box with multiple text boxes and radio buttons should appear in the central window, as seen in Figure 14.4.13. In the first box, titled Molecular Weight, type in the numbers `0` and `300`. In the second box, titled LOGP, enter the numbers `3.4` and `4.2`. In the third box, titled Drug Category enter the name `antidepressant`. Leave the Drug Type selection to **Approved Drugs** (the default). Press the Submit button at the bottom of the box. This process has effectively allowed the user to create the following query: *"find all drugs less than 300 da with LogPs between 3.4 and 4.2 that are antidepressants."*

    In this fine-tuning phase of the query, the first box allows the user to define the molecular weight range (0 to 300 da), the second box allows one to define the LogP range (3.4 to 4.2), and the third box allows the drug category (antidepressants) to be identified.

19. This query should generate a table with three drugs, including desipramine, doxepin, and bupropion. The table lists their DrugBank accession code and generic name, along with their molecular weight, logP, and drug category (Fig. 14.4.14). The user may choose to explore the different drugs in more detail by clicking on any of the hyperlinks. This will open the DrugCard for that particular drug. Many other complex queries can be constructed with the Data Extractor. For instance, try constructing a query that finds all drugs that target small (<500 Da) molecules.

    Steps 16 to 19 provide a brief overview of how to use the Data Extractor. The Data Extractor is perhaps the most powerful search utility in DrugBank. Unfortunately, it is also the least frequently used. This may stem from its somewhat more complex interface compared to the simple interfaces used for the Text Query or Search DrugBank for query tools. Nevertheless, with practice, one can use the Data Extractor to perform some very useful analyses of the data contained within DrugBank.

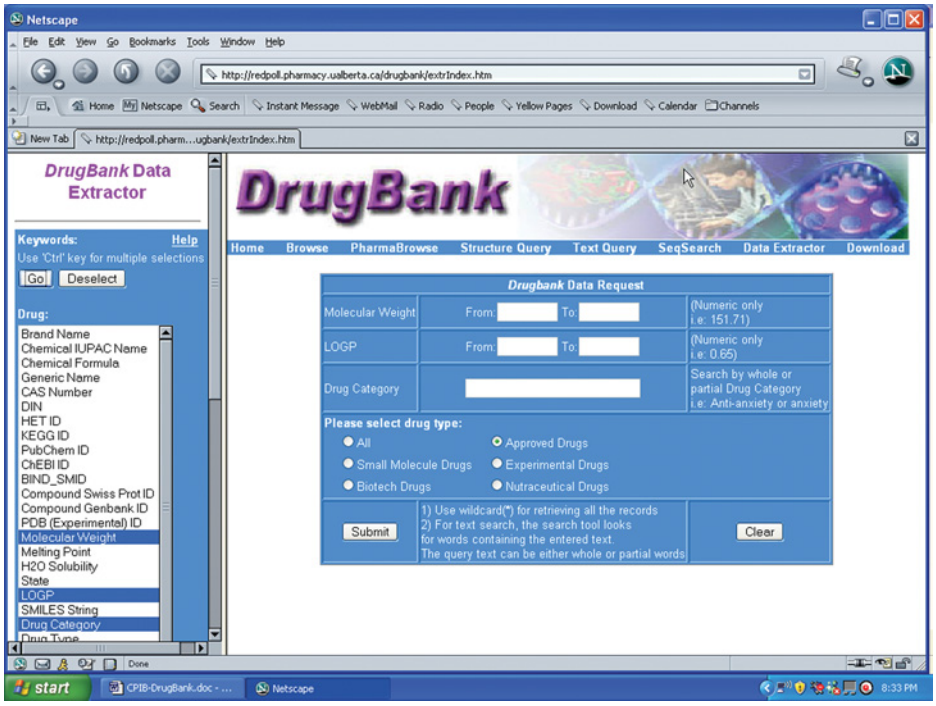

Figure 14.4.13 A screen shot of the Data Extractor query window. Users are required to fill in the text boxes using the suggested characters or formats.

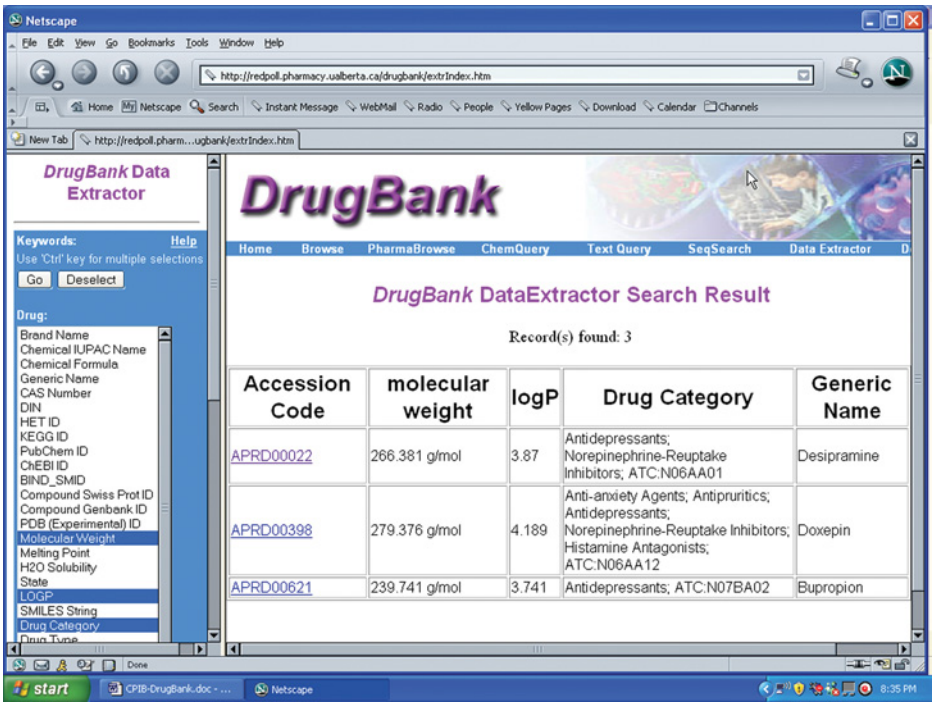

Figure 14.4.14 A screen shot of the output from a Data Extractor Query aimed at finding all drugs less than 300 Da with LogPs between 3.4 and 4.2 that are antidepressants.

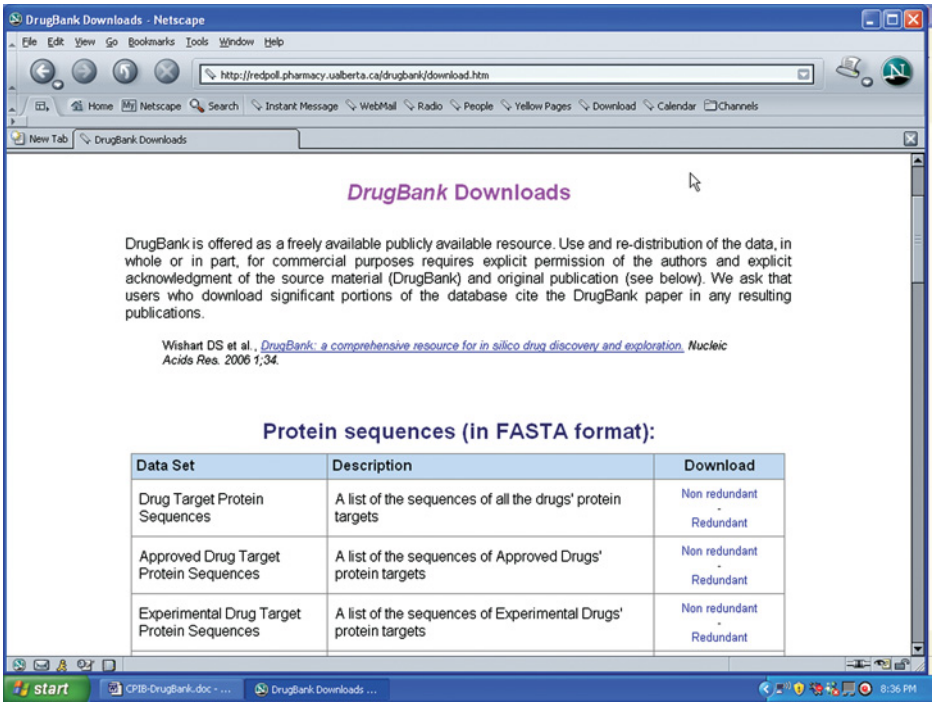
DrugBank Downloads - Netscape
DrugBank Downloads
DrugBank is offered as a freely available publicly available resource. Use and re-distribution of the data, in whole or in part, for commercial purposes requires explicit permission of the authors and explicit acknowledgment of the source material (DrugBank) and original publication (see below). We ask that users who download significant portions of the database cite the DrugBank paper in any resulting publications.
Wishart DS et al., DrugBank: a comprehensive resource for in silico drug discovery and exploration. Nucleic Acids Res. 2006 1;34.
Protein sequences (in FASTA format):
Data Set
Description
Download
Drug Target Protein Sequences
A list of the sequences of all the drugs' protein targets
Non redundant - Redundant
Approved Drug Target Protein Sequences
A list of the sequences of Approved Drugs' protein targets
Non redundant - Redundant
Experimental Drug Target Protein Sequences
A list of the sequences of Experimental Drugs' protein targets
Non redundant - Redundant

Figure 14.4.15 A screen shot of DrugBank's Download page.

Using the Download search option

20. Go to the DrugBank menu at the top of the page again and click on the **Download** hyperlink. The window shown in Figure 14.4.15 should appear. The Download page contains numerous large text files including DrugBank flat files, drug structure files, as well as redundant and nonredundant sequence files (both protein and DNA) for different classes of drug targets and drug metabolizing enzymes. Any or all of these files can be downloaded and further analyzed by interested users. The Download page also displays statistics about DrugBank (i.e., numbers of drug types, numbers of drug targets, numbers of nonredundant sequences). These flatfiles and statistics are updated every 3 to 6 months.

BASIC PROTOCOL 2

CHEMICAL STRUCTURE SIMILARITY SEARCHING

In cheminformatics, chemical structure similarity searching is the chemical equivalent of searching a sequence database for sequence homologs or searching the Protein Data Bank (PDB) for similar 3-D protein structures. It is particularly useful for organic chemists or natural product chemists who are interested in determining whether a newly synthesized compound or a newly identified natural product exhibits some similarity to a known drug. Chemical structure similarity searching is also useful for searching for compounds that may have the same parent compound or belong to the same drug class. Given the complexities or spelling inconsistencies found in many drug and chemical names, chemical structure similarity searching is often a useful search alternative. Indeed queries using chemical structures are often simpler and sometimes far more informative than text queries. This protocol describes how users may use several different features in DrugBank to search for chemically similar structures.

Necessary Resources

Hardware

Computer with Internet access.

Software

An up-to-date Internet browser, such as Internet Explorer (*http://www.microsoft.com/ie*); Netscape (*http://browser.netscape.com*); Firefox (*http://www.mozilla.org/fire*fox); or Safari (*http://www.apple.com/safari*). The Web browser must be capable of handling Java Applets (i.e., equipped with a Java interpreter) and capable of opening or viewing PDF files.

Files

None

Using the ChemQuery link

1. Go to the DrugBank Web site at *http://redpoll.pharmacy.ualberta.ca/drugbank/*.

   The DrugBank home page should be visible as should the blue menu bar located near the top of the page with eight clickable titles: Home, Browse, PharmaBrowse, ChemQuery, Text Query, SeqSearch, Data Extractor, and Download.

2. Click on the **ChemQuery** link. After a few seconds a window should appear with two pull-down tabs along with the ACD ChemSketch Java applet (Fig. 14.4.16). The top pull-down menu (**Search DrugBank Via**) allows users to select the type of chemical structure search (via Chemical Structure, Chemical Formula, Molecular Weight, or SMILES String). The lower pull-down menu (**Select Drug Type**) allows users to select the group of drugs to be searched (All Compounds, Approved Drugs, Experimental Drugs, Biotech Drugs, Small Molecule Drugs, and Nutraceuticals).

The ChemQuery tool is designed to permit a range of chemical structure queries. Using the Search DrugBank Via pull-down menu, users can either draw chemical structures into the ChemSketch applet, enter a range of molecular weights (through text boxes), type in a SMILES string (Weininger, 1988), or enter a chemical formula with a range of numeric indices. Searches via chemical formulas or molecular weight ranges are particularly useful for identifying drugs or drug structures via mass spectrometry (MS), where mass ranges or approximate chemical formulas (via FT-MS) are typically generated. Searches via SMILES strings or chemical structures are generally most useful for organic and natural product chemists, as well as many biochemists. SMILES (Simplified Molecular Input Line Entry Specification) is a specification for unambiguously describing the structure of chemical molecules using short ASCII strings. SMILES strings can be imported by most molecule editors for conversion back into 2-D drawings or 3-D molecular models. Recently, the IUPAC has introduced InChI notation as a standard for formula representation. However, SMILES is generally considered to have the advantage of being slightly more human-readable than InChI. DrugBank contains both InChI and SMILES representations for almost all of its small molecule drugs.

Drawing a chemical structure using ChemQuery

This particular part of the protocol will use the ChemQuery link to look for molecules that are similar to a certain tricyclic structure recently isolated from sea snails. Therefore, the user should leave the pull-down menus with their default selections (**Chemical Structure** and **Approved Drugs**).

3. To begin drawing the chemical structure, go to the top panel of buttons in the ChemSketch applet and press the button that looks like a stack of file cards (3rd button from right). This is the ChemSketch template library button. A small, mostly empty window should appear with a list on the left side containing different structure template names (**Rings, Chains, Bicyclics**, etc.) as seen in Figure 14.4.17.

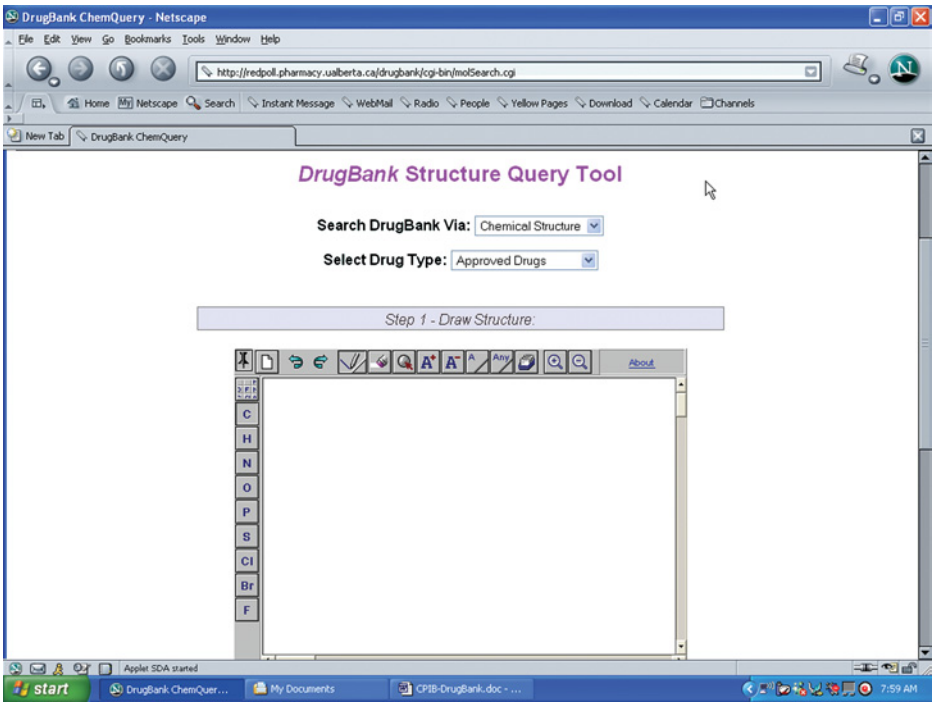

Figure 14.4.16 A screen shot of the ChemQuery window with the ACD ChemSketch Java applet at the center. This is where the query chemical structures can be drawn.

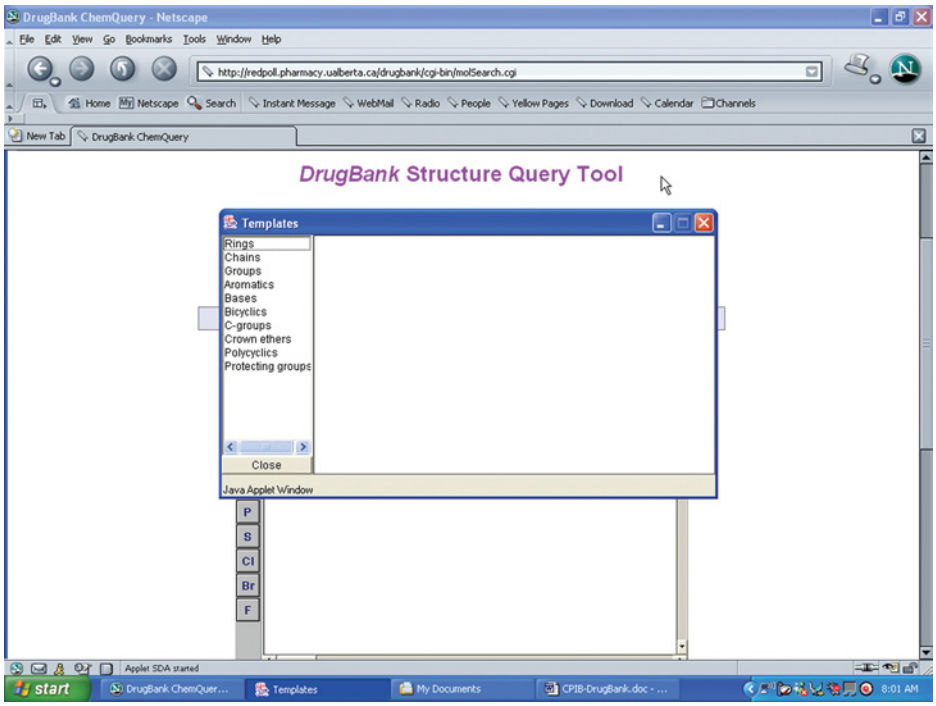

Figure 14.4.17 A screen shot of the ChemSketch applet with the template library window placed above the drawing palette.

The ChemSketch applet is a relatively simple chemical drawing utility. On the left side of the applet is a vertical list of Element buttons covering the most common elements or atoms used in organic chemistry. Above the C (carbon) button is a button for the periodic table of elements, which allows selection of rarer atoms. On the top of the applet is a series of buttons for drawing, erasing, moving, magnifying, undoing, redoing, or clearing different bonds or drawings. Mousing over each of the buttons will generate a one or two word description of what the button does (this appears on the right corner of the applet). Clicking on the About hyperlink on the right corner will launch a new window that describes the ChemSketch applet in more detail. Because many drug or drug-like structures are quite complex, probably the easiest route to drawing them via ChemSketch is to make frequent use of the template library and to add on molecular groups as needed.

4. Select the **Rings** template gallery by clicking on the word Rings at the top of the template list. A collection of 13 cyclic structures should appear. Go to the left side of this template gallery and click on one of the corners of the heptacyclic (seven-membered) ring. Upon clicking on the corner of the structure the template window should disappear. This action selects and copies the template image. Now, go to the ChemSketch drawing screen and click somewhere in the center of the screen. The clicking action pastes the heptacyclic ring image on to the ChemSketch drawing screen. The image shown in Figure 14.4.18 should now be seen.

5. Go back to the template button (the one with the stack of file cards) and click on it one more time. The template window should appear again. Select the **Aromatics** template by clicking on the word Aromatics at the top of the template list. A collection of 11 aromatic ring structures should appear in a new window. On the left corner of this template gallery is a benzene ring. Click on one of the bonds or edges (do not click on the atoms or vertices) of the ring. Now, go to the heptacyclic ring structure displayed on the ChemSketch palette and click on one its bonds (on the left side of the ring). This action pastes the previously selected benzene ring onto the seven-membered

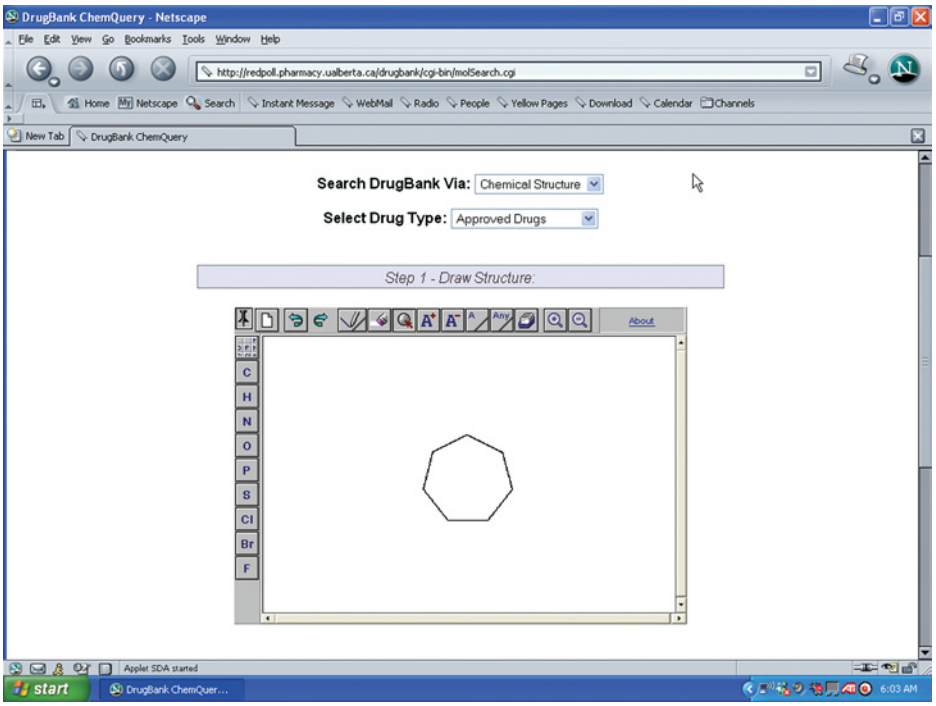

Figure 14.4.18 A screen shot of the ChemSketch applet with a heptacyclic ring placed at the center of the palette.

ring. Now go to the right side of the heptacyclic ring and click on one of the bonds opposite to where the previous benzene ring was pasted. A tricyclic structure with two benzene "mouse ears" should now be seen (Fig. 14.4.19).

If a mistake is made, the last operation can be undone by clicking on the curved-arrow button (the undo button; displayed in cyan on screen) which is the third button on the upper left corner. To start over, click on the second button (with a folded paper icon) on the upper left corner. This clears the screen. A common error in drawing structures using ChemSketch's template tools is to join two template molecules inappropriately. This may arise by clicking the atom of a template molecule and then clicking the bond of the molecule being rendered (or vice versa). This will join a corner to an edge, thereby creating an undesirable and unrealistic structure.

6. Go to the **Element buttons** on the left side of the window and click on the **N** atom (third atom button down). Now, click on the single free vertex (the top atom) in the heptacyclic ring that is between the two benzene rings. This will insert a nitrogen atom into the heptacyclic ring.

7. Click on the **Template button** again and select **Chains** (second word from top). A template gallery containing 12 aliphatic chains of different lengths will be displayed. Choose the pentane (five-carbon) chain and click on the left terminal atom. The template gallery window should disappear. Now, click on the NH atom that was just placed in the heptacyclic ring (Step 6). An aliphatic chain should now be attached to your tricyclic ring (Fig. 14.4.20). This is the structure of the compound isolated from sea snails.

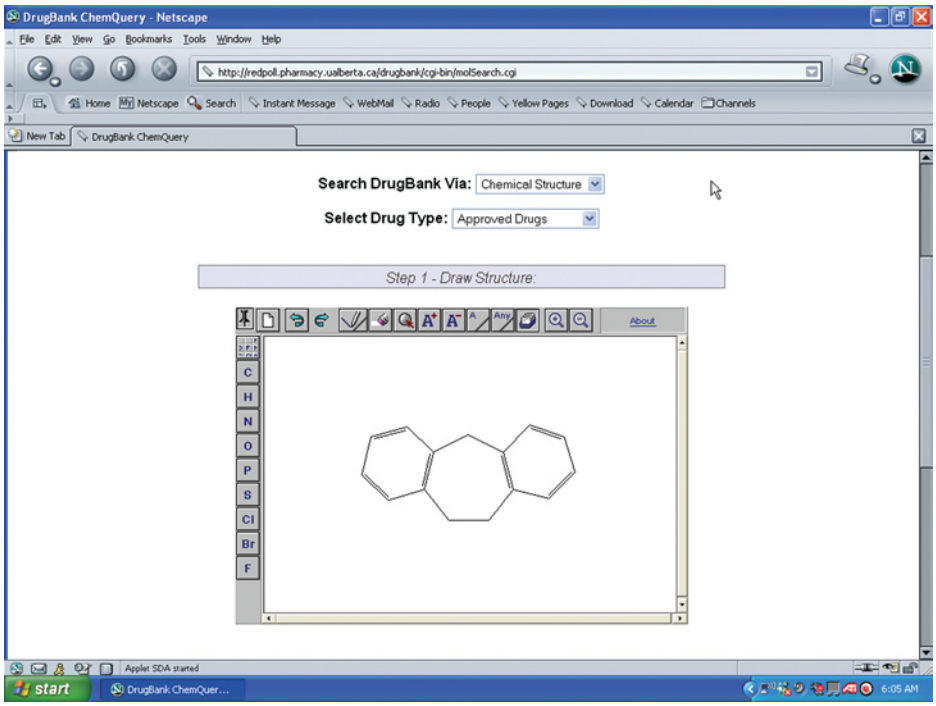

Figure 14.4.19 A screen shot of the partially completed tricyclic structure of the sea snail product.

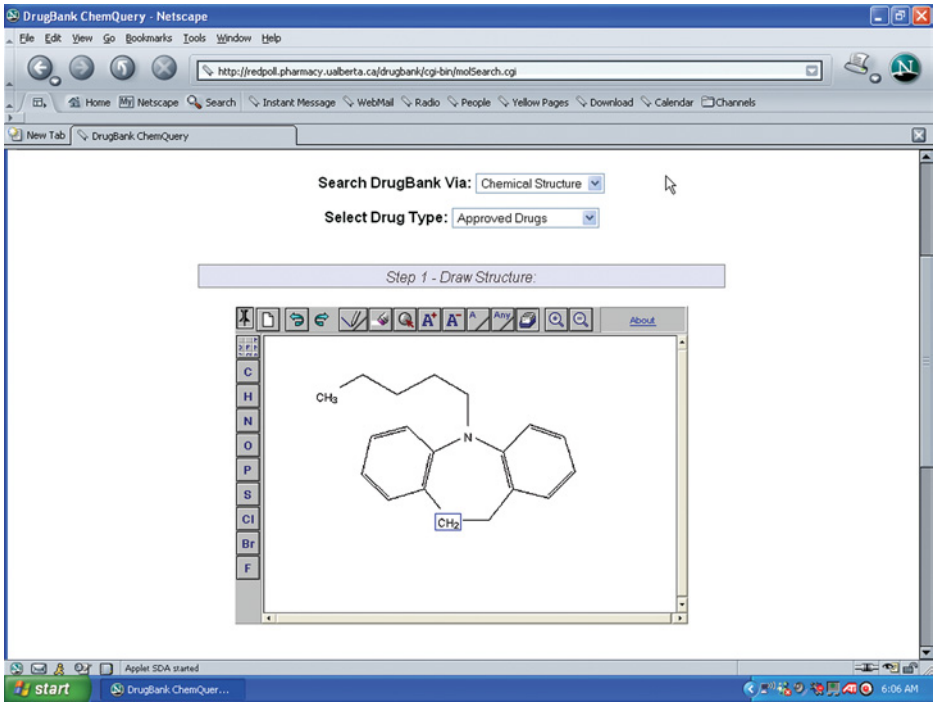

Figure 14.4.20 A screen shot of the complete chemical structure of the sea snail product. This is the query structure used by ChemQuery.

8. Scroll down the ChemQuery page and click on the button called **CLICK TO CONVERT TO MOL FILE**. Clicking this button will generate a MOL file that will automatically be pasted into the text box below the button (Fig. 14.4.21).

   The MOL file conversion allows users to cut/copy/paste the structure they just generated into a text document for future storage or reference. It also allows the conversion program called Babel to more easily convert the image that has just been drawn to a SMILES string.

9. Scroll down the ChemQuery page a little further and click on the button called **CLICK TO SUBMIT QUERY**. Within a few seconds, the ChemQuery window will be replaced with a new window displaying a list of similar compounds with the most chemically similar compounds listed at the top. The list format is essentially identical to that seen for the DrugBank Browser. The one difference is that, in the leftmost column, the matching score of each hit is indicated. Higher scores indicate better matches. Notice that this structure matches the structure of a number of well-known antidepressants, including desipramine (Fig. 14.4.22).

   This action takes the MOL file just generated and converts it to a SMILES string. The SMILES string is then compared using a specially developed text parsing program against all other SMILES strings in DrugBank. This text search is similar to a "Find text" query or a simplified sequence alignment. The ChemQuery search engine looks for shared chemical substructures by looking for shared SMILES substrings. A heuristic scoring method is used to prioritize and rank substring matches and to generate an overall chemical matching score. Clicking this button, a MOL file will be generated and pasted into the text box below the button.

Using the SMILES String option from ChemQuery

This protocol has described the steps for performing a graphical structure query search in DrugBank. However, as mentioned earlier (Step 2), ChemQuery also supports chemical structure queries using SMILES strings only.

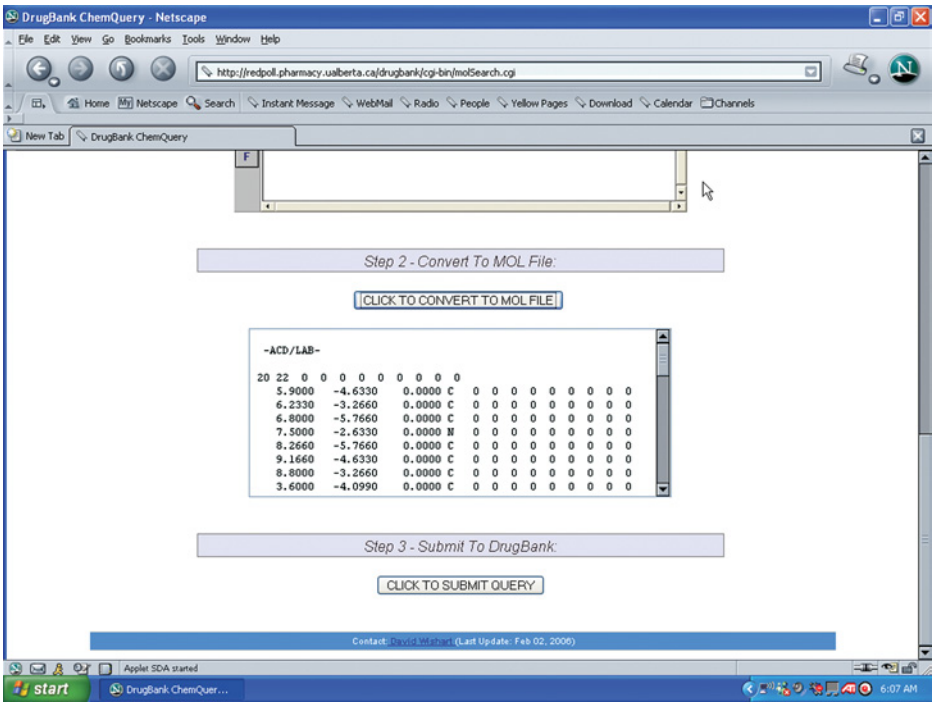
DrugBank ChemQuery - Netscape
File Edit View Go Bookmarks Tools Window Help
http://redpoll.pharmacy.ualberta.ca/drugbank/cgi-bin/molSearch.cgi
Home Netscape Search Instant Message WebMail Radio People Yellow Pages Download Calendar Channels
New Tab DrugBank ChemQuery
Step 2 - Convert To MOL File:
CLICK TO CONVERT TO MOL FILE
-ACD/LAB-
20 22 0 0 0 0 0 0 0 0 0
5.9000 -4.6330 0.0000 C 0 0 0 0 0 0 0 0 0
6.2330 -3.2660 0.0000 C 0 0 0 0 0 0 0 0 0
6.8000 -5.7660 0.0000 C 0 0 0 0 0 0 0 0 0
7.5000 -2.6330 0.0000 N 0 0 0 0 0 0 0 0 0
8.2660 -5.7660 0.0000 C 0 0 0 0 0 0 0 0 0
9.1660 -4.6330 0.0000 C 0 0 0 0 0 0 0 0 0
8.8000 -3.2660 0.0000 C 0 0 0 0 0 0 0 0 0
3.6000 -4.0990 0.0000 C 0 0 0 0 0 0 0 0 0
Step 3 - Submit To DrugBank:
CLICK TO SUBMIT QUERY
Contact: David Wishart (Last Update: Feb 02, 2006)
Applet SDA started
start
DrugBank ChemQuer...
6:07 AM

Figure 14.4.21 A screen shot of the MOL file generated by the ChemQuery conversion utility.

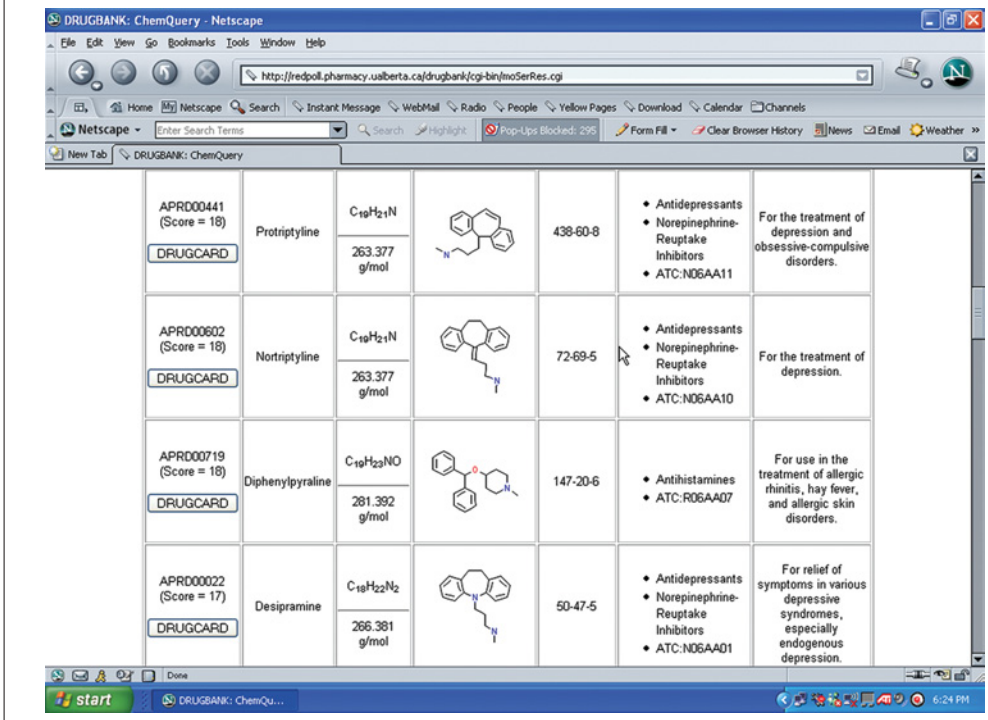

Figure 14.4.22 A screen shot of the similar structures found using the ChemQuery search tool.

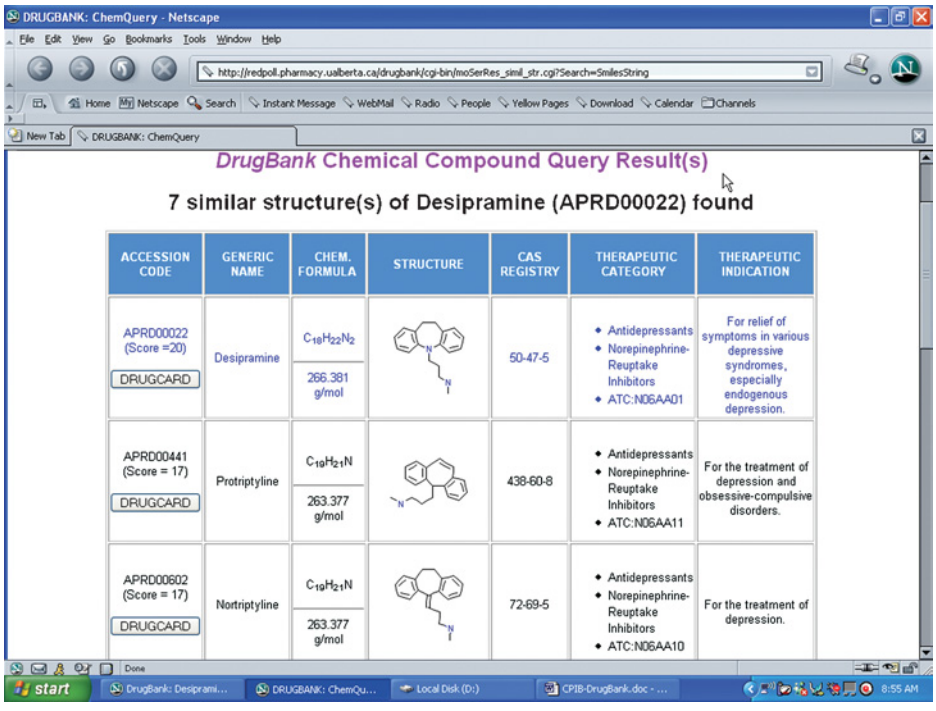

Figure 14.4.23 A screen shot of the table generated when the Search for Similar Structures button is pressed for desipramine.

10. In this case users should select the **SMILES String** option (bottom of the list) from the Search DrugBank Via pull-down menu. If one is adept with generating SMILES strings or already has a SMILES string for a given compound, then this option is generally much faster than drawing a structure.

11. In addition to ChemQuery, chemical structure searches are also possible through DrugBank's DrugCards. At the top of every DrugCard (Fig. 14.4.3), immediately above the **Creation Date** field and to the right is a button called **Show Similar Structures**. Clicking on this button will return a DrugBank Browser list displaying the most similar structures and their similarity scores (Fig. 14.4.23). Users may choose to have the search conducted over **Approved Drugs** (the default) or any of the other five drug categories (All Compounds, Approved Drugs, Experimental Drugs, Biotech Drugs, Small Molecule Drugs, or Nutraceuticals). This is a particularly useful option for researchers interested in understanding, comparing, or displaying the chemical modifications found in a given class of drugs.

IN SILICO DRUG TARGET IDENTIFICATION

BASIC PROTOCOL 3

In silico drug target identification is a method by which a protein or a set of proteins is involved in identifying protein sequences from a newly sequenced pathogen that exhibit some similarity to the sequences of known drug targets. Presumably, if a novel virus or a newly identified pathogenic bacterium share some significant sequence similarity to a protein that is a known drug target from another organism, then the same (or similar) drugs may be used to treat this pathogen. Alternately, these previously known drugs may serve as potential drug leads for developing more effective therapies. This protocol describes how users may use DrugBank's SeqSearch utility to identify potential drug targets from a small retrovirus.

Necessary Resources

Hardware

Computer with Internet access.

Software

An up-to-date Internet browser, such as Internet Explorer (*http://www.microsoft.com/ie*); Netscape (http://browser.netscape.com); Firefox (*http://www.mozilla.org/firefox*); or Safari (*http://www.apple.com/safari*). The Web browser must be capable of handling Java Applets (i.e., equipped with a Java interpreter) and capable of opening or viewing PDF files.

Files

A list of viral protein sequences is located at: *http://cpicanada.org/bioinfo2006/* (click on the **Virus** hyperlink). No other files are needed for this protocol.

1. Start your local Web browser and go to the DrugBank Web site at *http://redpoll.pharmacy.ualberta.ca/drugbank/*.

   The DrugBank home page should be visible as should the blue menu bar located near the top of the page with the 8 clickable titles Home, Browse, PharmaBrowse, ChemQuery, Text Query, SeqSearch, Data Extractor, and Download.

2. Click on the **SeqSearch** link. A window with the title DrugBank BLAST Search should appear (Fig. 14.4.24). As seen in the figure, the window contains a standard online BLAST search form with a text box window, **Submit** and **Reset** buttons as well as pull-down menus offering a choice of **Programs** (BLASTP or BLASTN), **Databases** (14 choices), and scoring **Matrices** (BLOSUM or PAM). Below the

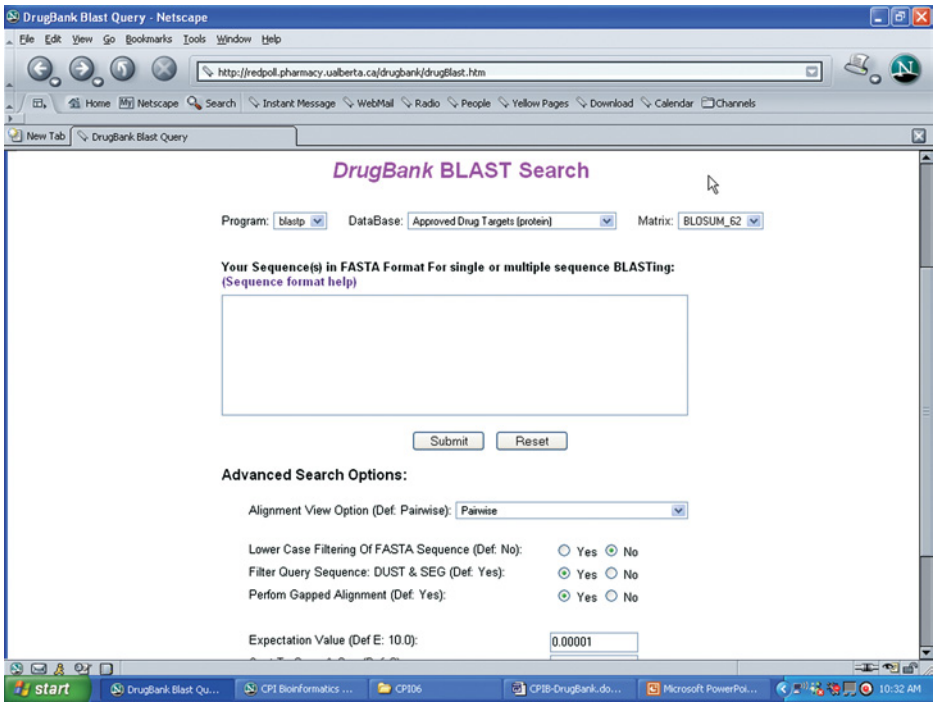
DrugBank Blast Query - Netscape
DrugBank BLAST Search
Program: blastp
DataBase: Approved Drug Targets (protein)
Matrix: BLOSUM_62
Your Sequence(s) in FASTA Format For single or multiple sequence BLASTing:
(Sequence format help)
Submit
Reset
Advanced Search Options:
Alignment View Option (Def: Pairwise): Pairwise
Lower Case Filtering Of FASTA Sequence (Def: No): Yes No
Filter Query Sequence: DUST & SEG (Def: Yes): Yes No
Perfom Gapped Alignment (Def: Yes): Yes No
Expectation Value (Def E: 10.0): 0.00001

Figure 14.4.24 A screen shot of the SeqSearch window. Note the standard BLAST query interface.

Submit and Reset buttons are textboxes and radio buttons for selecting various **Advanced Search Options**. In almost all cases users can leave everything (except the **Database** selection) in their default position.

A unique feature of the SeqSearch program is its capacity to handle multiple FASTA-formatted sequences. This allows users to BLAST multiple sequences—or even entire proteomes—using only a single paste and then clicking Submit. The required format of the multi-FASTA sequence list is described in more detail by clicking on the Sequence format help hyperlink above the text box. Note that the spacing between protein sequences does not matter (0, 1, or multiple spaces are allowed). Note also that the default Expectation Value for the BLASTP search is 0.000001 (1×10^{-6}) and not 10 (as is the usual default with GenBank searches). This is done to ensure the hits found in this search are significant enough to be considered truly homologous.

3. For this example the user will be looking for potential drug targets to a newly isolated retrovirus. To obtain the set of sequences to paste into the SeqSearch text box, launch a new browser window and go to *http://cpicanada.org/bioinfo2006/*. Click on the Virus hyperlink. A list of 16 viral sequences should be visible (Fig. 14.4.25). Select all 16 sequences by clicking and dragging through the window with your mouse. Copy the sequences (using the Copy option on your browser or using Ctrl+C).

4. Now click on the SeqSearch browser window to activate it and paste the sequences into the SeqSearch text box by clicking your mouse in the text box and using the Paste option on your browser (or Ctrl+V). The image shown in Figure 14.4.26 should now be seen.

Sixteen different protein sequences from the newly sequenced retrovirus have now been pasted. Use the scroll bars on the right side of the text box to see if all 16 sequences are there. Note that each sequence is separated by a ">" sign and a sequence title (Sequence 1, Sequence 2, etc.).

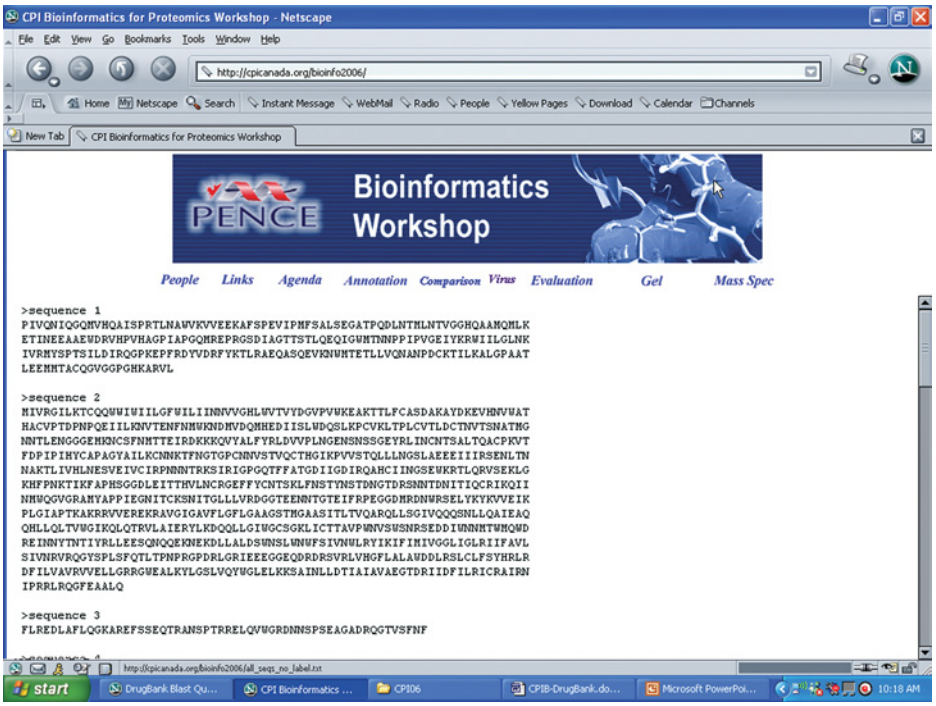

Figure 14.4.25 A screen shot of the Web site listing the 16 protein sequences for the newly sequenced retrovirus.

5. Press the Submit button. Within a few seconds, the BLAST search for all 16 input sequences should be completed. The program will return a concatenated, text-based BLAST summary for each of the 16 proteins that were submitted. The top portion of the SeqSearch output consists of a summary of the submitted sequences. Below that is the BLAST result for the first sequence (Sequence 1, with 231 residues). The output should indicate `***** No hits found ******`. Scrolling further down, the output for Sequence 2 should be seen (Fig. 14.4.27).

   This 143 residue protein exhibits about 74% sequence identity to the HIV envelope protein (or a portion thereof). Four hits are listed in the matching sequence list, meaning that the query sequence found matches to four other proteins in the DrugBank drug target database (all of which appear to be identical HIV proteins). Also displayed in this list is the name of the drug enfuvirtide and a hyperlink to the enfuvirtide drugcard.

6. Click on the hyperlink BIOD00106 listed beside the word enfuvirtide. The DrugCard for enfuvirtide or Fuzeon will appear. This page describes the drug and its mode of action in detail and it suggests that enfuvirtide may be able to target this viral protein target as well.

7. Scroll down further through the SeqSearch output page and look for other sequences that exhibit hits to known DrugBank targets and for drugs that would be likely to work on these protein targets. The user should find that most of the 16 proteins in this virus appear to be potential drug targets and that multiple existing drugs could be effective against it.

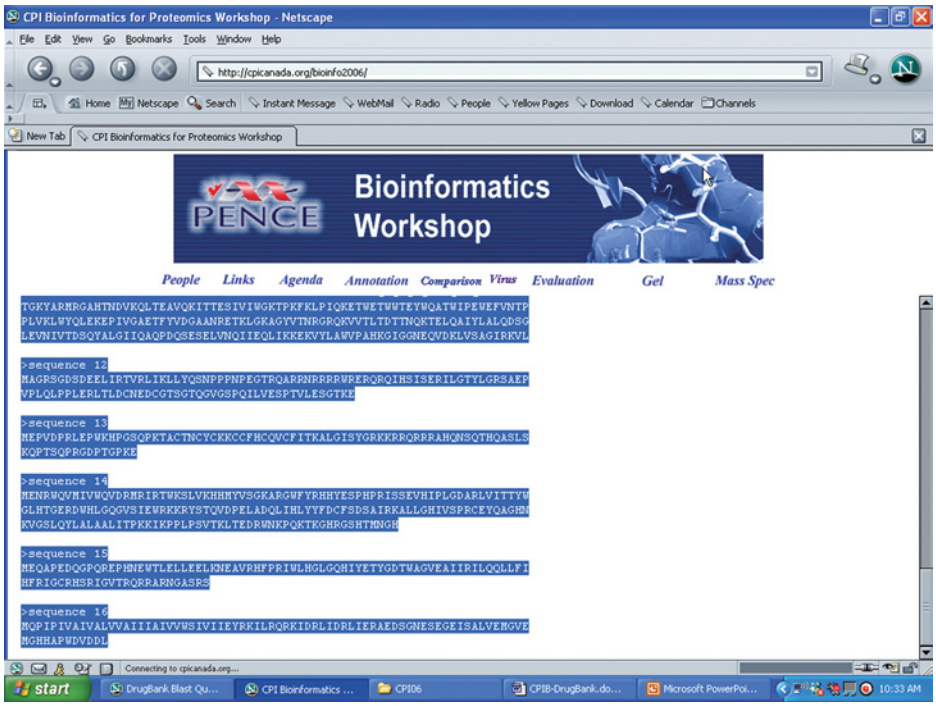

Figure 14.4.26 A screen shot of the SeqSearch window with all 16 retroviral protein sequences pasted into the text box.

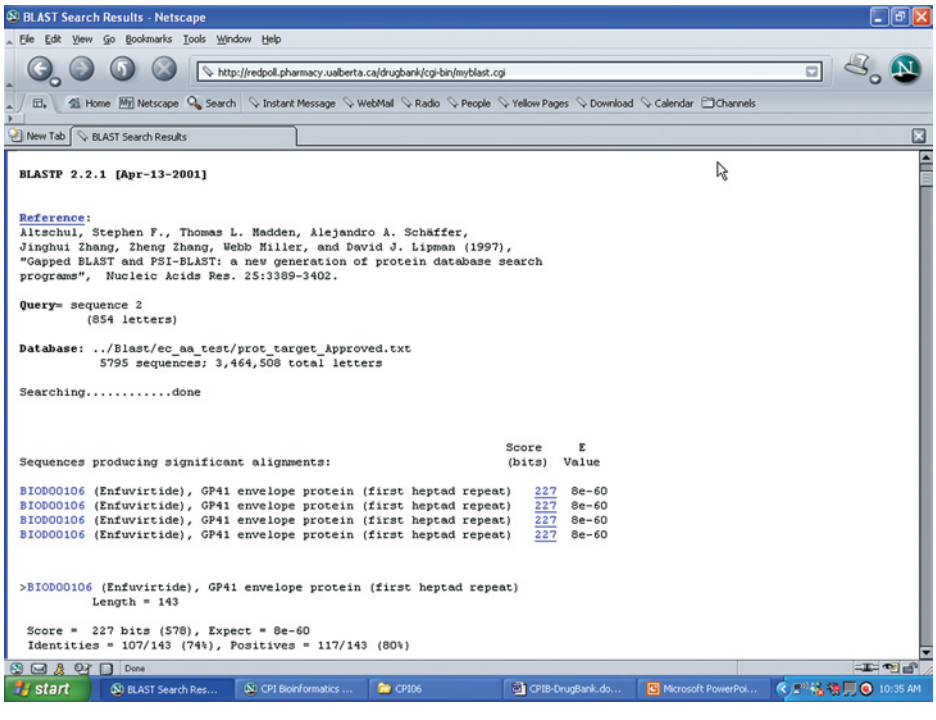
BLAST Search Results - Netscape
http://redpoll.pharmacy.ualberta.ca/drugbank/cgi-bin/myblast.cgi
BLASTP 2.2.1 [Apr-13-2001]
Reference:
Altschul, Stephen F., Thomas L. Madden, Alejandro A. Schäffer,
Jinghui Zhang, Zheng Zhang, Webb Miller, and David J. Lipman (1997),
"Gapped BLAST and PSI-BLAST: a new generation of protein database search
programs", Nucleic Acids Res. 25:3389-3402.
Query= sequence 2
(854 letters)
Database: ../Blast/ec_aa_test/prot_target_Approved.txt
5795 sequences; 3,464,508 total letters
Searching.....done
Sequences producing significant alignments:
Score (bits) E Value
BIOD00106 (Enfuvirtide), GP41 envelope protein (first heptad repeat) 227 8e-60
BIOD00106 (Enfuvirtide), GP41 envelope protein (first heptad repeat) 227 8e-60
BIOD00106 (Enfuvirtide), GP41 envelope protein (first heptad repeat) 227 8e-60
BIOD00106 (Enfuvirtide), GP41 envelope protein (first heptad repeat) 227 8e-60
>BIOD00106 (Enfuvirtide), GP41 envelope protein (first heptad repeat)
Length = 143
Score = 227 bits (578), Expect = 8e-60
Identities = 107/143 (74%), Positives = 117/143 (80%)

Figure 14.4.27 A screen shot of the SeqSearch output for the second sequence (Sequence 2) in the retroviral proteome. Note the drug names and hyperlinks in the output.

GUIDELINES FOR UNDERSTANDING RESULTS

Basic Protocol 1

This particular protocol was designed to show users how to explore the DrugBank database and to learn about a given drug (desiparmine), related drugs (tricyclic antidepressants), and its drug targets, along with other data about this class of drugs. The intent is to give users a broad overview of the data content and the capabilities of DrugBank. To summarize, Steps 1 to 2 provide brief a description of the DrugBank home page and its text search tool. Steps 3 to 9 take the user through a tour of a standard DrugCard, highlighting the layout, content, and important visualization and display tools. Steps 10 to 13 give a brief description of how to use the DrugBank Browser and PharmaBrowse tools, while Steps 14 to 15 demonstrate how the **Text Query** tool can be used. Steps 16 to 19 show how the Data Extractor can be used to extract far more specific and detailed information about certain drugs or drug classes, while Step 20 highlights the content and information that can be obtained from DrugBank's Download section. Overall, it is hoped that this protocol provides sufficient grounding and rationale to allow users to more fully explore DrugBank on their own. It is also worth noting that this protocol did not cover every aspect of DrugBank's search and query capabilities. In particular, the **ChemQuery** and **SeqQuery** options or their potential applications were not discussed. These query tools were discussed in separate protocols (Basic Protocols 2 and 3).

Basic Protocol 2

This protocol outlines the procedures for using DrugBank's chemical similarity search routines. Steps 1 to 2 outline the options available in DrugBank's ChemQuery tool, including mass range and chemical formula searches. These search tools are particularly useful for analytical chemists where compounds are frequently identified on the basis of their mass or chemical composition. Steps 3 to 9 describe how to use DrugBank's chemical structure drawing tools, specifically its ChemSketch Java applet. This series of steps illustrates how ChemQuery can be used to find known drugs that are structurally related to a natural product isolated from sea snails. Finally, steps 10 to 11 outline some of the other available chemical structure search utilities available in DrugBank, including the **Show Similar Structures** button that is available at the top of every DrugCard. This feature allows users to identify other compounds in DrugBank that are structurally similar to their drug of interest. This kind of query is particularly useful for researchers wishing to do comparative or rational drug design.

The results shown in Figure 14.4.22 demonstrate both the advantages and disadvantages of working with structure-based queries in DrugBank. Many drug discovery efforts are based on screening large libraries of organic compounds or on testing large numbers of natural product extracts, isolated from plants, soil bacteria, or sea creatures. When a bioactive substance is found and the structure is determined, the structure can often provide a rationalization for the compound's action. In this case, it can be seen that the sea snail compound drawn in steps 3 to 9 would be predicted to have some antidepressant activity and therefore it could be useful in treating depression or obsessive-compulsive disorders. It also exhibits some structural similarity to antiemetics, so it may be useful to treating motion sickness or sea sickness. These predictions, of course, would have to be verified by further wet-bench experiments. Other applications of this kind of structure-based search include the identification of potential protein targets (obtained by clicking on the DrugCard hyperlink), the prediction of unintended side effects (if a drug that is an antidepressant also exhibits structural similarity, say, to a diurectic), and the determination of whether a compound of interest may exhibit unexpected interactions with unintended protein targets.

It is worth noting that ChemQuery's structure similarity search did not find all tricyclic antidepressants and that the antiemetic compounds it found are only vaguely similar to the sea snail compound (Fig. 14.4.22). This result demonstrates one of the algorithmic limitations of the ChemQuery search. Since the program only uses SMILES strings and SMILES substrings as part of its query process, it can sometimes miss structurally similar compounds. Furthermore, because SMILES strings depend on the atom ordering in the MOL file, the sequence in which one draws the query molecule in ChemSketch can change the syntax of the SMILES string. As a result, the scoring and ordering of compounds generated via a ChemQuery structure search of a known drug will differ than the scoring and ordering of compounds generated via a Show Similar Structures search. To address these problems, the ChemQuery search algorithm makes use of molecular weight, chemical formula, and identified chemical functionalities as part of its scoring scheme. More sophisticated structure search tools are available that use graph theory (subdirected graph isomorphisms) and structural superpositioning to identify similar compounds. PubChem, in particular, offers an excellent online structure query tool that employs these techniques. An improved version of ChemQuery's structure search tool should be available in the Fall of 2007.

Basic Protocol 3

This short example demonstrates how the SeqSearch tool in DrugBank can be used to rapidly identify potential drugs and drug targets for a viral pathogen. As it turns out the particular virus used in the protocol exhibits a high degree of similarity to HIV and so there are a number of drugs that are likely to be effective against it. The principle behind this kind of drug target identification is relatively simple. All that is required is a comprehensive list of the sequences of known drug targets. By performing a sequence similarity search of an unknown or newly generated sequence (or set of sequences) against this database of known drug targets, it should be possible to identify if this sequence could be a likely drug target too. The unique aspect about DrugBank (and SeqSearch) is that it is currently the only electronic database that has a comprehensive collection of known drug target sequences (5795, at last count). Because DrugBank also links sequence information to drug information, the name of the drug (or drugs) that would be most effective against the drug target is also provided in its SeqSearch output.

Obviously, any potential hits generated by SeqSearch are only hypothetical. Confirmation of their utility as drug targets or the efficacy of the existing drug against the protein would have to be done using carefully controlled biochemical experiments or biological assays. Nevertheless, given the rate at which new viral and bacterial genomes are being sequenced (about two per day) and the enormous effort required to experimentally screen for potential drugs or drug targets, the use of a simple in silico tool like SeqSearch could be of enormous help.

To make the analyses relatively simple, this particular example was done only for a small virus. Certainly larger proteomes, including bacterial proteomes, could also be done. One difference between working with bacteria and viruses is the fact that not all proteins in a bacterium are essential whereas, for a virus, just about every protein is essential. As a rule, the most effective drug targets are those proteins that are highly conserved and are metabolically essential. Typically, only 200 to 300 proteins are absolutely essential in any given bacterium. Therefore, if one is trying to identify a set of potential drug targets for a pathogenic bacterium, it is usually a good idea to limit the search to those proteins that are metabolically essential. Current lists of essential genes for a number of bacteria is contained in the Database of Essential Genes (Zhang et al., 2004). This collection can be used concurrently with SeqSearch to identify (through homology) the most likely drug targets.

The identification of potential drug targets within the human genome (or proteome) is also possible with SeqSearch. However, as most human genes have already been identified and analyzed, the likelihood of finding a novel drug target through this sequence search method is rather remote. Instead, the greatest utility for this type of search for human drug targets may lie in the identification of unexpected or potentially co-inhibited proteins that were not the intended or known targets of a given drug.

COMMENTARY

Background Information

Overcoming the "two solitudes" of cheminformatics and bioinformatics

Cheminformatics and bioinformatics have largely evolved along two separate, almost divergent paths. Most chemical compound databases were developed without the intention or expectation that this information might be biologically or medically relevant. As a result, most chemical data is not linked in any meaningful way to protein names, protein targets, or their downstream physiological effects. Likewise, most sequence databases were developed without the intention of using this data to facilitate drug or drug-target discovery. As a result, most sequence data is not linked in any meaningful way to existing drug or disease information. This state of affairs largely reflects the "two solitudes" of cheminformatics and bioinformatics. Historically, neither discipline has really tried to integrate with the other. As a consequence, the wealth of electronic sequence/structure data that exists today has never been well-linked to the enormous body of drug or chemical knowledge that has accumulated over the past half century. This "information disconnect" is one of the reasons why bioinformatics has been so slow to help in the drug discovery and drug-target discovery process.

Attempts are now being made to remedy this situation. For instance, NCBI has now integrated OMIM (disease information), GenBank (sequence information), and PubChem (chemical or drug information) into its freely available Entrez Global Search Engine (Wheeler et al., 2005). Other efforts are also underway including GNF's Druggable Genome database (Orth et al., 2004) and the Therapeutic Target Database, or TTD (Chen et al., 2002). TTD is a freely accessible Web-based resource that contains linked lists of names for more than 1100 small molecule drugs and drug targets (i.e., proteins). It contains information about known protein and nucleic acid targets together with the associated disease conditions, pathway information, and the corresponding drugs/ligands directed to each drug target. Hyperlinks to other databases facilitate access to information regarding the function, sequence, 3-D structure, nomenclature, drug/ligand binding properties, and related literature about each protein/DNA target.

In addition to TTD, a number of comprehensive small molecule databases have also emerged including KEGG (Kanehisa et al., 2004), ChEBI (Brooksbank et al., 2005), and PubChem (Wheeler et al., 2005). Each contains tens of thousands of chemical entries—including hundreds of small molecule drugs. All three databases provide names, synonyms, images, structure files, and hyperlinks to other databases. Furthermore, both KEGG and PubChem support structure similarity searches. Unfortunately, these databases were not specifically designed to be drug databases, and so they do not provide specific pharmaceutical information or links to specific drug targets (i.e., sequences). Furthermore, because these databases were designed to be synoptic (containing fewer than 15 fields per compound entry), they do not provide a comprehensive *molecular* summary of any given drug or its corresponding protein target. In contrast to KEGG and PubChem, some of the more specialized drug databases, such as PharmGKB (Hewett et al., 2002) or online pharmaceutical encyclopedias such as RxList (Hatfield et al., 1999), tend to offer much more detailed clinical information about many drugs (their pharmacology, metabolism, and indications), but they were not designed to contain structural, chemical, or physico-chemical information. Instead their data content is targeted more towards pharmacists, physicians, or consumers—not drug-target discovery specialists.

DrugBank was developed to fill some of these database voids and to create a single, fully searchable in silico drug resource that links sequence, structure, and mechanistic data about drug molecules with sequence, structure, and mechanistic data about their drug targets. Fundamentally, DrugBank is a dual purpose bioinformatics-cheminformatics *knowledgebase* with a strong focus on

quantitative, analytic, or molecular-scale information about both drugs and drug targets. Knowledgebases can be distinguished from databases in that they contain not only facts and data, but also knowledge, i.e., the information or wisdom gained from a critical assessment of raw data. In fact, there is a growing trend in biology and medicine to enrich the textual or numerical content of many "first generation" databases, such as GenBank or the Protein Data Bank with detailed annotations and expert commentary to create "second generation" *knowledgebases* such as SwissProt or OMIM. In particular, DrugBank combines the data-rich molecular biology content normally found in curated sequence databases such as SwissProt and UniProt (Bairoch et al., 2005) with the equally rich data found in medicinal chemistry textbooks and chemical reference handbooks. By constructing comprehensive, meaningful links between drugs, diseases, and sequences, DrugBank can allow one to learn from past successes (and even past failures) in terms of what proteins make for good drug targets (soluble versus membrane-bound, structural proteins versus enzymes, and strong binders versus weak binders), what types of pathways (metabolic, signaling, nuclear, and cytoplasmic) make for good therapeutic intervention strategies, what characteristics in small molecules make for good drug leads (Lipinski's Rule of Five), what classes of drug targets are under- or over-represented in existing formularies and so on.

For example, using DrugBank it is relatively easy to determine that 96% of FDA-approved drug target types are peptide or protein molecules. Less than 1% of all drug targets are small molecules (i.e., adenosine, uric acid, digoxin, iduronic acid, asparagine, hyaluronic acid, etc.), while three classes of DNA (eukaryotic, prokaryotic, and viral) and two classes of RNA (bacterial rRNA and retroviral cRNA) serve as nucleic acid drug targets. Likewise, the vast majority of drug targets (97%) and drugs (89%) are associated with endogenous diseases, while only a tiny minority of drug targets (3%) and drugs (11%) are actually associated with exogenous or infectious diseases. Endogenous diseases are typically chronic human disorders or conditions that arise due to germ-line mutations (genetic diseases), somatic mutations (cancer), the aging process (atherosclerosis, immune disorders, etc.), or some other internal factors. Exogenous diseases are typically temporary diseases or conditions that arise from external, nonhuman agents such as viruses, bacteria, fungi, protozoans, poisons, or poisonous animals.

DrugBank is unique not only in the type of data it provides but also in the level of integration and depth of coverage it achieves. In addition to its extensive small molecule drug coverage, DrugBank is certainly the only public database that provides any significant information about the 110+ approved biotech (i.e., protein) drugs. DrugBank also supports an extensive array of visualizing, querying, and search options including a structure similarity search tool and an easy-to-use relational data extraction system. It is hoped that DrugBank will serve as a useful resource to not only members of the pharmaceutical research community but to educators, students, clinicians, and the general public.

Critical Parameters and Troubleshooting

To facilitate consistency and simplicity, DrugBank has very few user-settable parameters. Users may change the settings on the SeqSearch (i.e., local BLAST) search, but the default settings are generally sufficient for most applications. More details about the critical parameters for BLAST searches can be found in *UNITS 3.3 & 3.4*. The other component to DrugBank that can cause users some problems is the Data Extractor tool. This relational query system requires that the users know something about the content in different DrugBank fields, including number ranges (for MW or pKa) or type of textual content. Obviously, typing in a negative molecular weight, a misspelled or nonsense word, a number where a word is expected, or a word where a number is expected will cause some unpredictable behavior in the search engine. If a questionable result is generated or if a query seems to "hang" for >1 minute, users are requested to double-check their query to make sure it contains none of the above errors. Nonresponsiveness can be a problem with any Web site. This may reflect heavy use, periodic maintenance, server hardware problems, or the submission of an erroneously structured query that, in effect, searches and grabs all data in the database. DrugBank is heavily used and certainly its performance may be compromised by this heavy use (or abuse). If users experience consistent problems with either Web site access or program performance, they are encouraged to contact the DrugBank staff or the author of this unit.

DrugBank is a curated database, not an archival database. This means that the data in DrugBank are compiled, assessed, and entered by trained curators. Every effort is made to ensure the data in DrugBank is as correct, complete, and as current as possible.

However, as with any database, DrugBank contains some errors. These may be errors arising from data entry, drug de-accessioning (removing a drug but leaving the DrugBank link in place), or recent revisions to the knowledge about a particular drug or drug target. If a user believes they have identified an error, it is encouraged for them to contact the DrugBank staff as soon as possible. Usually, errors can be confirmed and corrected within a few days. Likewise users may find some data are missing in certain DrugCards. In many cases, the information (melting point, solubility, pKa, and drug target) has never been collected or is not yet known. However, if users become aware of a new source of information that fills in a missing data field, they are encouraged to contact the DrugBank staff.

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Internet Resources

http://redpoll.pharmacy.ualberta.ca/drugbank/
DrugBank Web site

http://pubchem.ncbi.nlm.nih.gov/
PubChem Web site

http://xin.cz3.nus.edu.sg/group/cjttd/TTD_ns.asp
TTD Web site

http://www.genome.jp/kegg/drug/
KEGG drug database Web site

http://www.cmpharm.ucsf.edu/~walther/webmol.html
WebMol Web site

http://tubic.tju.edu.cn/deg/
Database of Essential Genes

Contributed by David S. Wishart
University of Alberta and the National Institute of Nanotechnology (NINT)
National Research Council
Edmonton, Alberta Canada

Using ChemBank to Probe Chemical Biology

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ABSTRACT

ChemBank (*http://chembank.broad.harvard.edu/*) is a public, Web-based informatics environment. ChemBank stores and makes freely available data derived from small molecules and small-molecule screens and has resources for relating and studying these data. Currently, ChemBank stores information on hundreds of thousands of small molecules and hundreds of biomedically relevant assays performed at the Broad Institute screening center. Web-based analysis tools are available within ChemBank to study the relationships between small molecules, cell measurements, and cell states. This unit demonstrates the use of ChemBank data to ask and answer questions relating to chemical biology and screening experiments contained within ChemBank. *Curr. Protoc. Bioinform.* 22:14.5.1-14.5.26. © 2008 by John Wiley & Sons, Inc.

Keywords: chemical biology • cheminformatics • data analysis • database • high-throughput screening • small molecule

INTRODUCTION

ChemBank (*http://chembank.broad.harvard.edu/*) stores information on small molecules and biomedically relevant assays that have been performed at the Broad Institute screening center. The ChemBank Web-based user interface makes it easy to retrieve this information, and the ChemBank online help pages (*http://chembank.broad.harvard.edu/details.htm?tag = Help*) provide descriptions of the Web pages and the data displayed. This unit provides a brief introduction to ChemBank, followed by a series of scenarios that show how one might use ChemBank to address specific research questions. Each scenario is an independent hands-on tutorial. The collection of scenarios introduces most of the features in ChemBank (version 2.1.3).

Basic Protocols 1 and 3 will explore the intersection of biological annotations within small-molecule records in ChemBank and the performance of small molecules in high-throughput screens (HTS). Basic Protocols 2, 4, and 5 will focus on using chemical structure manipulation and comparison to answer research questions. Basic Protocol 6 describes how to download and export data from ChemBank for use in other software applications.

Note that data are regularly added to ChemBank, and that addition of data may alter some of the expected output of steps within the protocols contained in this unit.

MAKE A HYPOTHESIS ABOUT THE POTENTIAL BIOLOGICAL ACTIVITY OF A MOLECULE

BASIC PROTOCOL 1

In this protocol, imagine that a compound ("Compound X") has been synthesized and that one is looking for clues about the potential biological activities of the molecule. Because the small-molecule structure is known, the SMILES string, structure, or ChemBankID for the molecule is available. Using ChemBank, it is possible to explore the biological

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activity of structurally similar molecules scoring as standard hits in assays, to explore the assays in which these molecules appear, and to generate hypotheses about potential biological activities of Compound X.

This protocol will use the known SMILES string of Compound X to find molecules in ChemBank that are structurally similar to Compound X and that would score as standard hits in any assay. A multi-assay heatmap is used to visualize the CompositeZ scores for these molecules across all assays in which they were tested. From the heatmap, it is possible to view the ChemBank annotations for a molecule. Where the annotations include activity-related terms from the scientific literature, one can use the heatmap to identify the assay(s) in which the molecule scored as a hit. The heatmap also provides access to a description of the project and the assay.

For more information about the definition of a "hit" and information about ChemBank in general, refer to Background Information at the end of this unit and Seiler et al. (2008). Briefly, the calculated CompositeZ score is the overall measure of whether a compound scored as active in an assay. In ChemBank, the term "hit" refers to a non-zero response based on a researcher's subjective criteria. The term "standard hit" refers to a defined cutoff for the CompositeZ score and Reproducibility based on the objective criteria. For most assays, these criteria are |CompositeZ| > 8.53 AND |Reproducibility| > 0.99, where CompositeZ and Reproducibility are both calculated values stored by ChemBank.

Necessary Resources

Hardware

A computer with a minimum of 256 Mb of RAM, connected to the Internet. A high-speed Internet connection (e.g., DSL or cable modem) is recommended, as dial-up connections will likely be exceedingly slow to load ChemBank Web pages and visualizations.

Software

A Web browser such as Internet Explorer, Firefox, or Safari is required to access ChemBank

NOTE: For this example, the SMILES string for Compound X is: CCn1cc(C(=O)O)c(=O)c2c(N)c(F)c(N3CCNCC3)c(F)c12.

Find the molecule of interest

1. Go to the ChemBank home page: *http://chembank.broad.harvard.edu/welcome.htm*. Under Find Small Molecules in the menu bar on the left-hand side of the screen (see Fig. 14.5.1), click the link for "by user list."

   ChemBank displays the "Search by user list" page (also illustrated in Fig. 14.5.1). This page may also be directly accessed by going to the URL http://chembank.broad.harvard.edu/chemistry/search/input/userList.htm.

2. Enter the SMILES string (see Necessary Resources, above) in the "names or SMILEs" text box. Instead, if the ChemBankID is known, it can be entered here. Click the "search now" button.

   ChemBank displays the search results in list format (Compound Search page, not illustrated in Fig. 14.5.1).

   If a SMILES-based search does not return a ChemBank molecule, click "by similarity" under Find Small Molecules in the left-hand menu bar and enter the SMILES string on the "Search by similarity" page. Identify the molecule of interest among those returned by the similarity search.

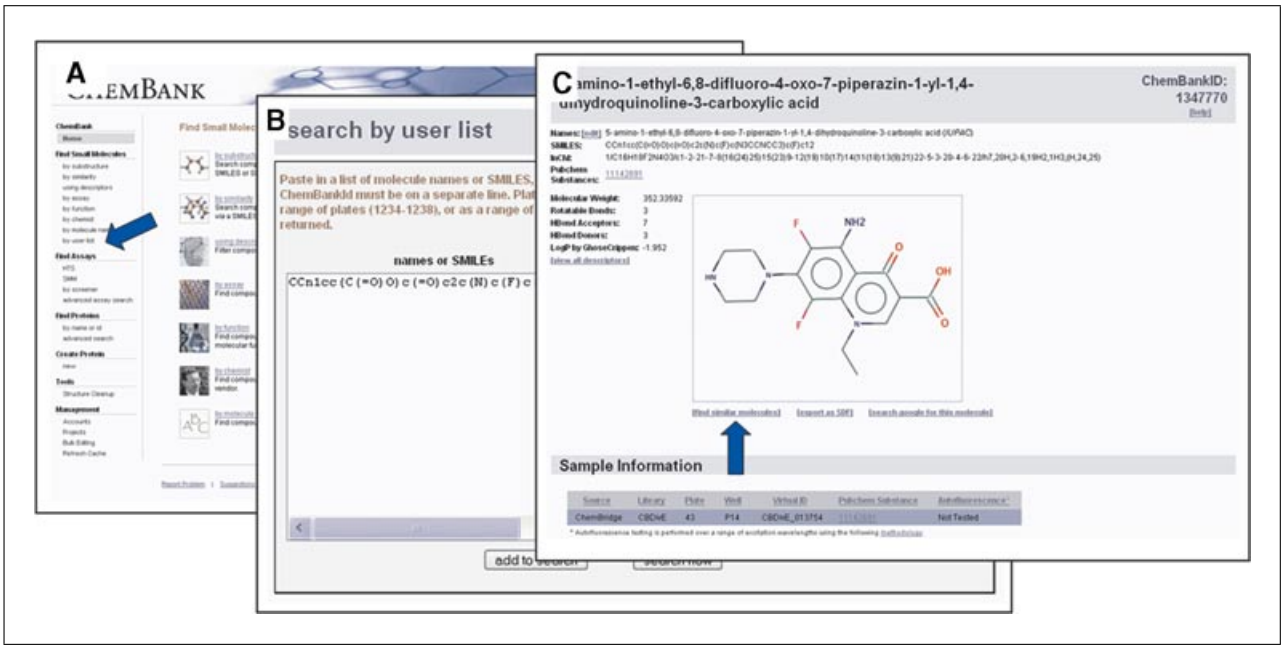

Figure 14.5.1 Screenshots of steps to search for a molecule by SMILES representation using ChemBank similarity search. (**A**) ChemBank home page with search menus at far left. (**B**) Illustration of SMILES input in the "search by user list" feature. (**C**) ChemBank Molecule Display page associated with the search result from (B); the arrow in (C) shows how to search for structurally similar entries directly from the Molecule Display Web page.

Find molecules similar to the molecule of interest

For this example, focus on the molecule with ChemBankID "1347770."

3. On the Compound Search page containing the search results obtained at step 2, click ChemBankID "1347770" to bring up the Molecule Display page (illustrated in the right-hand panel of Figure 14.5.1).

4. Click "[find similar molecules]" (below the structure depiction) to search for related compound structures.

   ChemBank displays the search results in a list format.

Modify the search to find molecules that are similar to the molecule of interest AND that scored as standard hits in any assay

5. Click "[modify]" (near the top of the page under the number of molecules found) to modify the query.

   ChemBank displays the "molecule search builder" page (not illustrated in the figures).

6. From the drop-down list labeled "Select a criterion to add," select Assay and then click the "add" button.

   ChemBank displays the "Search by assay" page (not illustrated in the figures).

7. Select all projects and assays by clicking "Check all."

   By default, ChemBank finds molecules that scored as standard hits in the selected assays.

8. Click the "add to search" button to review the search criteria or the "search now" button to begin the search.

   ChemBank displays the search results in a list format (not illustrated in the figures).

Use a heatmap to visualize the screening results for these molecules

9. Click "[view multi-assay result heatmap]" from the row of links near the top of the screen.

   ChemBank displays the "select visualization features" page (not illustrated in the figures), which prompts the user to select the assays to display in the heatmap.

10. Select all projects and assays by clicking "Check all," then click the "generate visualization" button.

    ChemBank displays a heatmap that shows the molecules that were on the search result page and the assays in which they were tested. For additional description and a figure showing a heatmap, see Basic Protocol 6 and the Commentary.

    A molecule can be used in multiple assays and/or multiple wells in an assay; therefore, the heatmap may contain more compounds than there are molecules in the search results.

11. For a more readable heatmap, sort the compounds by name and plate number by using the bottom scroll bar to scroll all the way to the right side of the visualization, and then clicking the Compound column heading (on the right side of the heatmap).

Display the details of each compound, including activity-related terms (if any) from the scientific literature

For this example, focus on ciprofloxacin and norfloxacin.

12. Double-click a compound name to display the Molecule Display page. Scroll down this page and notice that both ciprofloxacin and norfloxacin include the term "antibacterial" under the Therapeutic Uses heading.

    The browser may need to allow popup windows for the ChemBank site to display this result properly; if a popup blocker is in use, temporarily turn it off.

Having found compounds of interest, use the heatmap to explore assays in which those compounds scored as standard hits

13. Scroll the heatmap display to scan for dark blue and dark red cells, which indicate the lowest and highest CompositeZ scores for the compounds of interest, ciprofloxacin and norfloxacin.

    Hover over a cell to display the associated compound name, assay name, and CompositeZ score.

14. When an assay is found in which the compound scored as a standard hit, double-click the assay name (at the top of the column) to display information about the assay and its associated project. A "standard hit" refers to a predefined cutoff for both CompositeZ score and Reproducibility and is represented as a dark red or dark blue cell on the heatmap. Other views, such as the platemap view, represent a Standard Hit with a marker dot. The precise definitions can be found in the Help section of the ChemBank Web site.

    Examining the descriptions of these assays/projects may provide insight into the molecule of interest.

    Ciprofloxacin and norfloxacin scored as standard hits in several assays. For example, both compounds scored as standard hits in project "907" (ciprofloxacin in assay "907.0134" and "907.0135" and norfloxacin in assay "907.0115") and ciprofloxacin scored as a standard hit in assay "1000.0052."

Having found compounds with annotations of interest, search ChemBank to find all compounds that have those annotations

15. Under Find Small Molecules in the left-hand menu bar, click the "by function" link. On the "Search by function" page, for Ontology, select Therapeutic Use and for

Term, enter `anti-bacterial`. Leave the "Include child term matches" check box checked (the default). Click the "search now" button to start the search.

ChemBank displays the search results in a list format (not illustrated in the figures).

Ontological terms are case-sensitive. If unsure of a term, use the Browse (magnifying glass) button to select it rather than simply typing it in.

16. Modify the search to find only those molecules that scored as standard hits (see steps 5 through 8).

ChemBank displays a subset of the original search results

All molecules with an activity annotation of "anti-bacterial" from the literature which scored as standard hits in an assay have now been found. See Figure 14.5.2.

17. To generate a list of all projects and assays, under Find Assays in the left-hand menu bar, click "advanced assay search." On the "advanced assay search" page which then appears, click the Search button. ChemBank displays a list of all assays and their associated projects. To save the list to a file, click "[export as text]" near the top of the page.

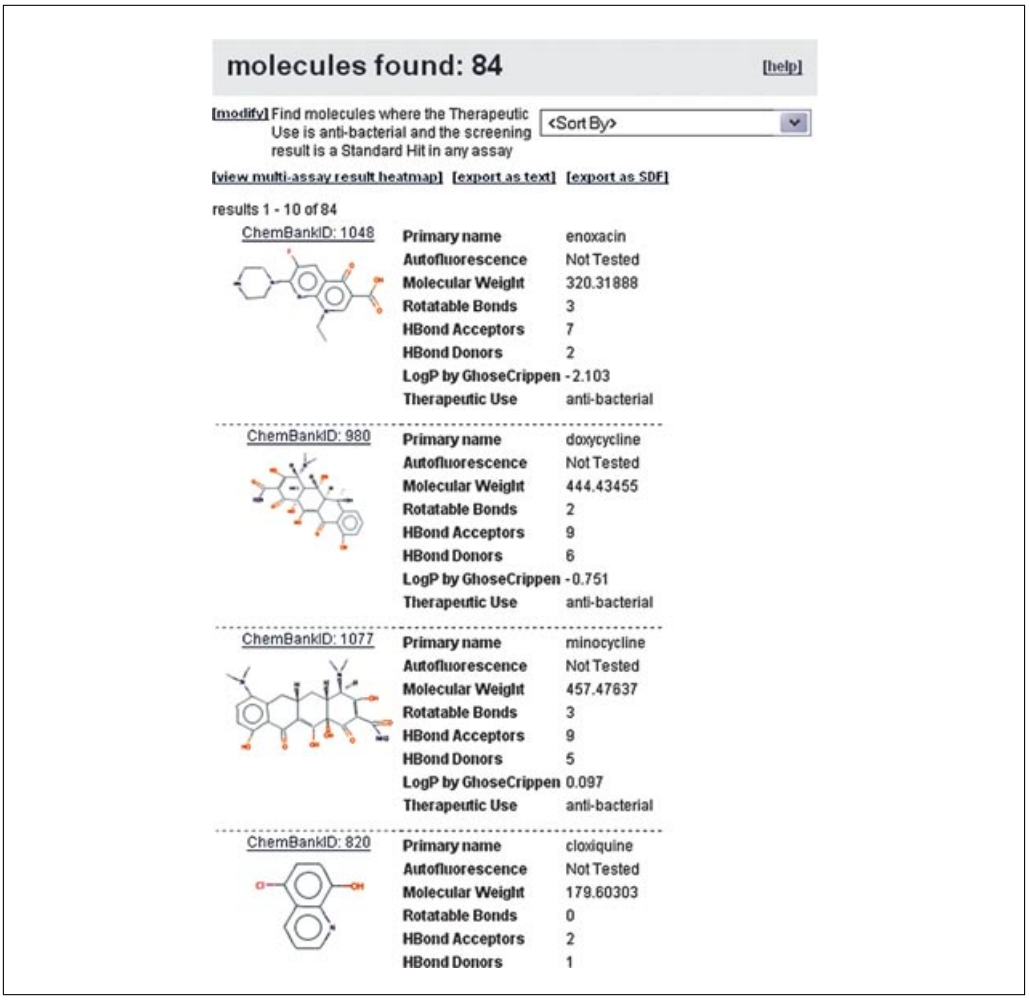

Figure 14.5.2 Screenshot of portion of search results for molecules scoring as "hits" in an assay with the biological annotation of "anti-bacterial."

SELECT TESTED COMPOUNDS ON WHICH TO PURSUE ADDITIONAL SCREENING OR FOLLOW-UP CHEMISTRY

For this protocol, the reader should imagine that he or she has observed that hundreds of compounds have been tested in a ChemBank project and would now like to select a small number of those compounds for additional screening or follow-up chemistry. A heatmap will be used to visualize the CompositeZ scores for compounds that were tested in the assays of this project. For any assay of interest, an assay histogram will be used to examine the CompositeZ scores for the tested compounds. From the histogram, the compounds with the most significant scores should be selected. For any compound of interest, an assay scatterplot is used to determine the replicate reproducibility of the compound. Having identified compounds active in this project based on significant CompositeZ scores, one then determines if the compounds are *selectively* active in this assay or are globally active in many assays. To do so, a heatmap should be generated to visualize the screening results for these compounds in *all* assays of *all* projects.

Necessary Resources

Hardware

A computer with a minimum of 256 Mb of RAM, connected to the Internet. A high-speed (e.g., DSL or cable modem) Internet connection is recommended, as dial-up connections will likely be exceedingly slow to load ChemBank Web pages and visualizations.

Software

A Web browser such as Internet Explorer, Firefox, or Safari is required to access ChemBank

NOTE: For this example, the project of interest is "DihydroorotateDehydrogenase."

Find compounds that scored as hits in the project of interest

1. Go to the ChemBank home page (*http://chembank.broad.harvard.edu/welcome.htm*). In the menu bar on the left-hand side of the page, click "view projects," scroll down the list of projects that appears, and then click "DihydroorotateDehydrogenase."

   ChemBank displays the View Project page (not illustrated in the figures).

   Notice that the project contains pairs of assays taken at 0- and 30-minute timepoints. The assays named "Calc. . ." show the change between the two timepoints, which are the values of interest. In looking at this project, the focus is on the "Calc. . ." assays.

2. Click "[find hits]" to find the compounds that scored as standard hits in the assays of this project.

   ChemBank displays the search results in list format.

Use a heatmap to visualize the molecules and the assays in which they were tested

Focus on the values of interest in this project by including only the "Calc. . ." assays of the "DihydroorotateDehydrogenase" project in the heatmap.

3. Click "[view multi-assay result heatmap]."

   ChemBank displays the "Feature selection" page, which prompts the user to select the assays to display in the heatmap.

4. In the Select Projects and Assays list box, click the plus (+) icon next to DihydroorotateDehydrogenase to display the assays in that project.

5. Select the calculated assays by clicking the checkbox next to each assay. For this example, select assays "Calc(E1-E2)(1021.0018)," "Calc(E1-E2)1021.0019," and

"Calc(E1-E2)1020.0020." Click the "generate visualization" button to display the heatmap.

ChemBank displays a heatmap that shows the molecules that were on the search page and the selected calculated assays in which they were tested.

6. Scroll the heatmap to scan for the dark red and dark blue cells that indicate the lowest and highest CompositeZ scores for these compounds.

   The dark blue cells indicate that the compounds scored as hits in assay "1021.0019" have the lowest CompositeZ scores.

Use an assay histogram to select the compounds with the lowest CompositeZ scores in assay "1021.0019"

7. Double-click assay "1021.0019" to display its details.

   The browser may need to allow popup windows for the ChemBank site to display this result properly; if a popup blocker is in use, temporarily turn it off.

8. Click "[view histogram]" near the top of the page to display a histogram of the CompositeZ scores for the assay (see Fig. 14.5.3).

9. Select the compounds with a CompositeZ score less than −20 by clicking on the histogram image at "−20" and dragging the cursor to the left side of the histogram (it does not matter where the cursor is on the vertical axis). ChemBank draws a box around the selected portion of the histogram.

   It is also possible to manually input values into the boxes to the left of the histogram image rather than drawing a box within the histogram.

10. Click "[view molecules in range as list]" to display the selected compounds on the search result page.

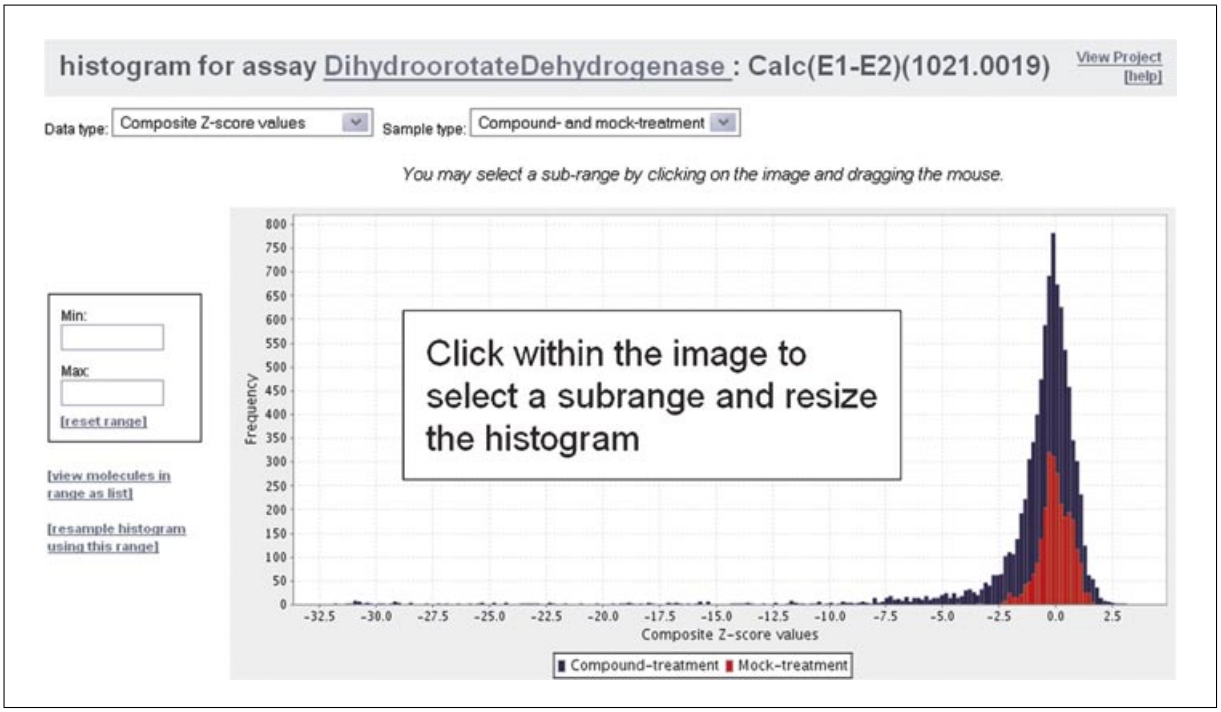

Figure 14.5.3 Screenshot of histogram of CompositeZ scores for the assay "DihydroorotateDehydrogenase: Calc(E1-E2)(1021.0019)." Mock treatment measurements are depicted in red; compound-treatment wells are depicted in blue. For the color version of this figure go to *http://www.currentprotocols.com*.

Use an assay scatterplot to determine the replicate reproducibility of a selected small-molecule test

11. Click a representative molecule from the list to display information about the molecule. For this example, click ChemBankID "3052589." A Molecule Display page appears.

12. The Screening Test Instances section of the Molecule Display page shows compound activity across all projects. Click the "CompositeZ score" heading once to sort the list by ascending CompositeZ scores.

    The assay of interest, "1021.0019," with its low CompositeZ score, moves to the top of the list.

13. Click assay "1021.0019," "DihydroorotateDehydrogenase: Calc(E1-E2)," to display its details.

14. Click "[view scatterplot]" to determine replicate reproducibility for the compound of interest, ChemBankID "3052589." The page that appears is shown in Figure 14.5.4.

    The plot shows the CompositeZ scores of both mock-treatment and compound-treatment wells from the selected assay. The score for the compound of interest is highlighted in cyan. In this example, most scores (including the highlighted score) lie on the diagonal, indicating similar results for both replicates.

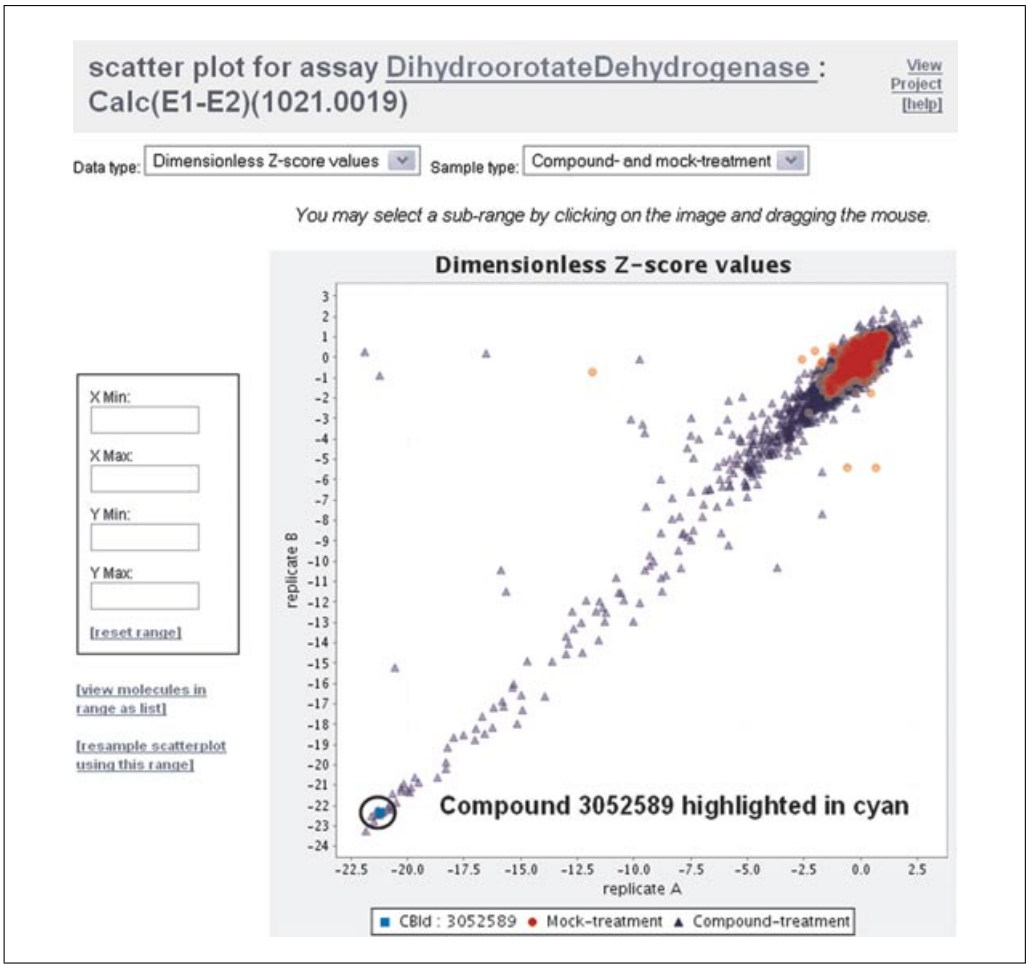

Figure 14.5.4 Screenshot of scatterplot of Dimensionless Z-score values (Seiler et al., 2008) for assay "1021.0019" in ChemBank. Mock-treatment values are in red circles; compound-treatment values are in blue squares; compound with ChemBankID "305289" highlighted in cyan. For the color version of this figure go to *http://www.currentprotocols.com*.

15. Optionally, check the replicate reproducibility of other molecules in the list.

Use a heatmap to determine whether the compounds active in this project are selectively active

16. Repeatedly click the Back button of the browser to return to the list of the molecules with lowest CompositeZ scores in assay "1021.0019" of the "DihydroorotateDehydrogenase" project (i.e., to the page that was obtained in step 10).

17. Click "[view multi-assay result heatmap]." ChemBank displays the "select visualization features" page, which prompts the user to select the assays to display in the heatmap. Select all projects and assays by clicking "Check all," then click the "generate visualization" button.

18. Scroll the heatmap to scan for the dark red and dark blue cells that indicate the lowest and highest CompositeZ scores for these compounds.

    The heatmap shows relatively restricted activity in projects other than project "1021," "DihydroorotateDehydrogenase."

DETERMINE WHICH SMALL MOLECULES MAY PERTURB BIOLOGICAL PATHWAYS AND PROCESSES

BASIC PROTOCOL 3

To understand the protocol below one should imagine studying a biological pathway or process and finding a related high-throughput screening (HTS) project in ChemBank (the biological object of the project is a cell line or an organism). ChemBank also contains screening projects for purified proteins—small-molecule microarray (SMM) projects (Duffner et al., 2007) and HTS projects for homogenous proteins. It may be desirable to identify assays related to the biological process under study, or SMM projects containing proteins known to be involved in that biological process. Searching for compounds that are active in both types of assays, the investigator hopes to identify small molecules that may affect the biological pathway or process being studied.

In this protocol, the ChemBank user finds compounds that scored as standard hits in both the HTS assay of interest and an SMM assay. From the Molecule Display page for a compound scoring in both types of assays, the screening test results are viewed; SMM assays in which the compound scored as a standard hit are identified and the View Assay page is used to examine the assay and the tested protein. The protocol also describes how to identify other HTS projects in which the compound scored as a standard hit and use a heatmap to examine the compound response pattern across the projects in which the compound is active.

Necessary Resources

Hardware

A computer with a minimum of 256 Mb of RAM, connected to the Internet. A high-speed Internet connection (e.g., DSL or cable modem) is recommended, as dial-up connections will likely be exceedingly slow to load ChemBank Web pages and visualizations.

Software

A Web browser such as Internet Explorer, Firefox, or Safari is required to access ChemBank

NOTE: For this example, the project of interest is "PSACAntagonistScreen."

Find hits in the "PSACAntagonistScreen" project

1. Go to the ChemBank home page (*http://chembank.broad.harvard.edu/welcome.htm*). In the menu bar on the left-hand side of the page, click "view projects," scroll down the list of projects that appears, and then click "PSACAntagonistScreen."

   ChemBank displays the View Project page (not illustrated in the figures).

2. Click "[find hits]" to find the compounds that scored as standard hits in the assays of this project.

   The ChemBank search returns thousands of molecules.

Modify the search to find compounds that scored as standard hits in PSACAntagonistScreen AND in a small-molecule microarray (SMM) project

3. Click "[modify]" to modify the query.

   ChemBank displays the "Molecule search builder" page (not illustrated in figures).

4. From the drop-down list labeled "Select a criterion to add," select Assay, and then click the "add" button.

   ChemBank displays the "search by assay" page.

5. From the Assay Type drop-down list, select "small-molecule microarray."

   ChemBank filters the list of projects and assays to display only small-molecule microarray projects.

6. For this example, select the small-molecule microarray project "SMM-DIV06Annotation." Click the "search now" button.

   ChemBank displays the search results in list format.

   Each molecule scored as a standard hit in the project of interest and as a standard hit in the small-molecule microarray project "SMMDIV06Annotation." For this example, focus on the molecule with ChemBankID "2144641."

7. Click ChemBankID "2144641" to display the molecule details. A Molecule Display page appears.

8. Click the "CompositeZ score" heading twice to sort the screening test instances by descending CompositeZ score. Locate a small-molecule microarray assay in which the compound scored as a hit. For this example, select the "SMMDIV06Annotation" assay with the CompositeZ score of 4.0887, "1066.0011."

9. Click the assay name, "ManualSNR(1066.0011)," to view assay details, including the name of the protein that was tested.

   ChemBank displays the View Assay page (not illustrated in figures).

10. Click the "protein components" link for more information about the protein.

    ChemBank displays the Protein page (not illustrated in figures).

11. Click the Back button of the browser to return to the Molecule Display page (i.e., the page obtained in step 7). Optionally, examine the other "SMMDIV06Annotation" assays and their proteins. To list the small-molecule microarray assays together, click the Assay Type heading to sort the screening test instances by assay type.

12. Click the Back button of the browser to return to the search results page (i.e., the page obtained in step 6).

    On the search results page, each molecule scored as a standard hit in the project of interest and in at least one screening assay for a purified protein. For a molecule of interest, use the Molecule Display page to find other HTS projects in which it scored as a hit. For this example, focus on the molecule with ChemBankID "2144641."

13. Click ChemBankID "2144641" to display molecule details. ChemBank displays the Molecule Display page.

14. Click the "CompositeZ score" heading to sort the screening test instances by descending CompositeZ score.

    In addition to scoring as a standard hit in the "PSACAntagonistScreen" and "SMMDIV06Annotation" projects, this compound also scored as a hit in the "FacioscapulohumeralMD," "HemeDetoxification," and "PKCoreAssaySet" projects.

Use a heatmap to examine the compound response patterns across these projects

15. Click the Back button of the browser to return to the search results page (i.e., the page obtained in step 6).

16. Click "[view multi-assay result heatmap]."

    ChemBank displays the "select visualization features" page, which prompts the user to select the assays to display in the heatmap.

17. In the Select Projects and Assays list of check boxes, select the following projects of interest: "FacioscapulohumeralMD," "HemeDetoxification," "PKCoreAssaySet," "PSACAntagonistScreen," and "SMMDIV06Annotation." Click the "generate visualization" button.

    ChemBank displays a heatmap that shows the molecules on the search result page and the assays (from the selected projects) in which they were tested.

18. Examine the assays in project "1066," "SMMDIV06Annotation."

    The response pattern for the "Porco-J/BostonU" compound (well "2111L03") is distinctly different from that of the "Neumann-C/Harvard" compounds (wells "2098E03," "2097G06," "2098G18," and "2098E08"). While all five compounds show reactivity in assays "1066.0001" and "1066.0002," only the "Porco-J/BostonU" compound shows consistently negative CompositeZ scores across the other assays in that project. To examine a compound, including its structure, double-click the compound name in the Compound column.

19. The response pattern for the "Neumann-C/Harvard" compounds appear to have potentially interesting similarities across the assays of project "1051" ("HemeDetoxification") and potentially interesting differences across the assays in project "1035" ("PSACAntagonistScreen"). For more information about an assay, double-click the assay number.

DISSECT SMALL-MOLECULE STRUCTURE USING ASSAY PROFILES

BASIC PROTOCOL 4

Imagine that a small molecule has been synthesized and that one is interested in examining the response patterns for structurally related compounds. Because this is a known molecule, the structure, SMILES string, or ChemBankID of the molecule is known.

In this protocol, the ChemBank user finds structurally related molecules by using the JME Molecular Editor (Ertl and Jacob, 1997) to draw the known molecular structure. The search is modified to find structurally related molecules that scored as standard hits in any assay. A heatmap is used to visualize the CompositeZ scores for these molecules across all assays. In the heatmap, the compounds are sorted by SMILES string to group structurally similar compounds. Small-molecule groups with similar and/or significantly different response patterns are identified and other molecules in ChemBank that share similar response patterns are found. Finally, a structure-data file (`.sdf`) for the compounds associated with a particular response pattern is generated.

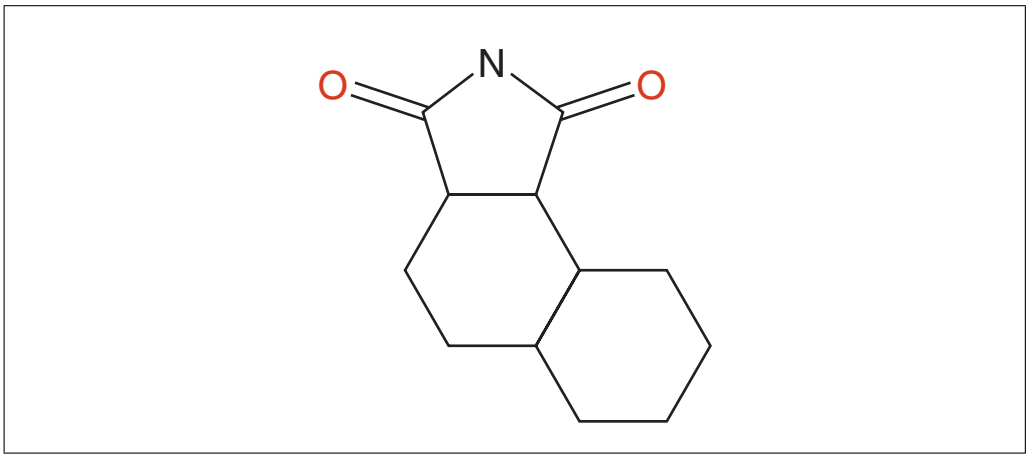

Figure 14.5.5 Structure to be drawn in the JME Molecular Editor (Ertl and Jacob, 1997) for Basic Protocol 2.

Necessary Resources

Hardware

A computer with a minimum of 256 Mb of RAM, connected to the Internet. A high-speed (e.g., DSL or cable modem) Internet connection is recommended, as dial-up connections will likely be exceedingly slow to load ChemBank Web pages and visualizations.

Software

A Web browser such as Internet Explorer, Firefox, or Safari is required to access ChemBank

A text editor is needed to view the `.sdf` output file and other software to view the chemical graphs encoded within the`.sdf`

NOTE: For this example, the known molecular structure is shown in Figure 14.5.5.

Find molecules structurally related to the molecule of interest

1. Go to the ChemBank home page (*http://chembank.broad.harvard.edu/welcome.htm*). Under Find Small Molecules, click the "by substructure" link. On the "search by substructure" page, use the JME Molecular Editor (Ertl and Jacob, 1997) to draw the molecular structure shown in Figure 14.5.5. Click the "search now" button to find molecules that share the structure.

   Figure 14.5.6 shows the "search by substructure" window with the drawn molecular structure. The SMILES string for this structure is: C12C(C(=0)NC1=0)CCC3C2CCCC3.

Modify the search to find molecules that contain this substructure AND that scored as standard hits in any assay

2. In the page displaying the search results, click "[modify]" to modify the query.
3. From the drop-down list labeled "Select a criterion to add," select Assay, then click the "add" button.

   ChemBank displays the "search by assay" page.

4. Select all projects and assays by clicking "Check all."

   By default, ChemBank finds molecules that scored as standard hits in the selected assays.

5. Click the "add to search" button to review the search criteria or the "search now" button to begin the search.

   ChemBank displays the search results in list format.

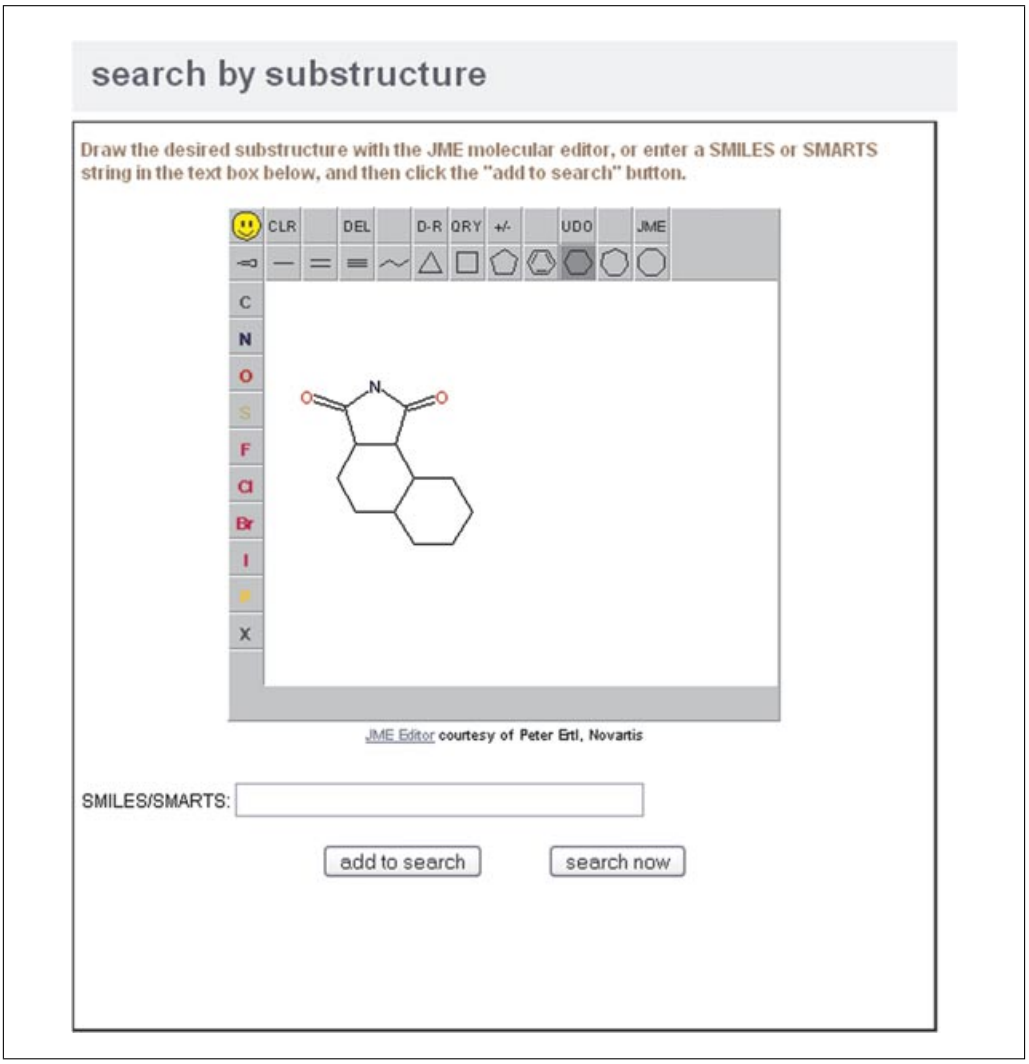

Figure 14.5.6 Screenshot of structure from Figure 14.5.5 drawn within the structure editor interface in ChemBank.

Use a heatmap to visualize the molecules on the search result page and the assays in which they were tested

6. On the page with the search results, click "[view multi-assay result heatmap]."

   ChemBank displays the "select visualization features" page, which prompts the user to select the assays to display in the heatmap.

7. Select all projects and assays by clicking "Check all," then click the "generate visualization" button.

   ChemBank displays a heatmap that shows molecules on the search result page and the assays in which they were tested.

8. Scroll the heatmap to scan for the dark blue and dark red cells that indicate the lowest and highest CompositeZ scores for these compounds.

   For this example, notice that the compound of interest is active in project "1012" and its compound response patterns vary across the assays in that project.

Examine project "1012"

9. From the heatmap, double-click an assay to display its information. For example, double-click assay "1012.0064."

The browser may need to allow popup windows for the ChemBank site to display this result properly; if a popup blocker is in use, temporarily turn it off.

ChemBank displays the View Assay page.

10. From the View Assay page, click the project name to display information about the project.

    ChemBank displays the View Project page for the "NOXSuperoxideGeneration" project.

Simplify the heatmap by displaying only the "NOXSuperoxideGeneration" project

11. Using the Back button of the browser, return to the "select visualization features" page (i.e., the page obtained in step 6), which prompts the user to select the assays to display in the heatmap.

12. Select the "NOXSuperoxideGeneration" project and click the "generate visualization" button.

    ChemBank displays a heatmap that shows the same set of compounds and the assays (in the "NOXSuperoxideGeneration" project) in which they were tested.

Group compounds by structure in the heatmap and examine their response patterns

13. Click the SMILES heading to sort the compounds by SMILES string.

    ChemBank sorts the compounds by ordering the SMILES strings alphabetically. This is a crude way of grouping compounds by structure similarity.

14. Notice that compounds "2110K11" and "2110K03" have similar response patterns: they both have high CompositeZ scores in assays "1012.0064" and "1012.0065" and have low scores in all other assays. Compound "2111M11" has a distinctly different response pattern: it has high CompositeZ scores in all assays.

15. For a visualization of the response pattern, select all assays by shift-clicking the assay numbers, and select compounds "2110K11," "2110K03," and "2111M11" by control-clicking the compound names, then select Profile from the View menu on the heatmap menu bar. When finished viewing the profile, close the profile window.

16. Double-click the compound name in the heatmap to display more detailed information, including its molecular structure and activity across assays in all projects. For example, double-click "2110K11." ChemBank displays the Molecule Display page.

17. Display the Molecule Display page for the three compounds of interest: "2110K11," "2110K03," and "2111M11." As one would expect, the first two have a similar structure, which is distinct from that of the third.

Find molecules that have a response pattern similar to compounds "2110K11" and "2110K03"and generate a structure-data file (`.sdf`) for those molecules

18. Return to the heatmap and examine the response pattern for "2110K11" and "2110K03." These compounds have CompositeZ scores greater than 5.4 in assays "1012.0064" and "1012.0065" and less than -1.5 in assays "1012.0068" and "1012.0069." Hover over a cell of the heatmap to see the CompositeZ scores.

19. Define the first search criterion: molecules that have CompositeZ scores "> 5.4" in assay "1012.0064":

    a. In the menu bar on the left-hand side of the heat map, under Find Small Molecules, click "by assay." On the "search by assay" page, select assay "1012.0064" from the "NOXSuperoxideGeneration" project.

    b. At the bottom of the page, use the drop-down list to select "molecules satisfying the condition" and then specify CompositeZ > 5.4 to complete the search criterion.

c. Click the "add to search" button.

ChemBank displays the "molecule search builder" page.

Projects can have many assays. On the "search by assay" page, use the Find function of the browser to find the correct assay.

20. Define the second search criterion: molecules that have CompositeZ scores > 5.4 in assay "1012.0065":

    a. From the drop-down list labeled "Select a criterion to add," select Assay and then click the "add" button.

    b. On the "search by assay" page, select assay "1012.0065" from the "NOXSuperoxideGeneration" project.

    c. At the bottom of the page, use the drop-down list to select "molecules satisfying the condition" and then specify CompositeZ > 5.4 to complete the search criterion.

    d. Click the "add to search" button.

21. Define the third search criterion: molecules that have CompositeZ scores < -1.5 in assay "1012.0068."

    a. From the drop-down menu labeled "Select a criterion to add," select Assay and then click the "add" button.

    b. On the "search by assay" page, select assay "1012.0068" from the "NOXSuperoxideGeneration" project.

    c. At the bottom of the page, use the drop-down menu to select "molecules satisfying the condition" and then specify CompositeZ<–1.5 to complete the search criterion.

    d. Click the "add to search" button.

22. Define the fourth search criterion: molecules that have CompositeZ scores < -1.5 in assay "1012.0069."

    a. From the drop-down menu labeled "Select a criterion to add," select Assay and then click the "add" button.

    b. On the "search by assay" page, select assay "1012.0069" from the "NOXSuperoxideGeneration" project.

    c. At the bottom of the page, use the drop-down menu to select "molecules satisfying the condition" and then specify CompositeZ<–1.5 to complete the search criterion.

    d. Click the "add to search" button.

23. Click the "search" button.

    ChemBank displays the two molecules from the heatmap. No other molecules tested in this project have this response pattern.

24. Click "[export as SDF]" to generate an `.sdf` file for these molecules.

Find molecules that have a response pattern similar to compound "2111M11" and generate a structure-data file (`.sdf`) for those molecules

25. Return to the heatmap (displayed in step 12) and examine the response pattern for "2111M11." This compound has CompositeZ scores greater than 4.7 in assays "1012.0064," "1012.0065," "1012.0066," "1012.0067," "1012.0068," and "1012.0069" of the "NOXSuperoxideGeneration" project.

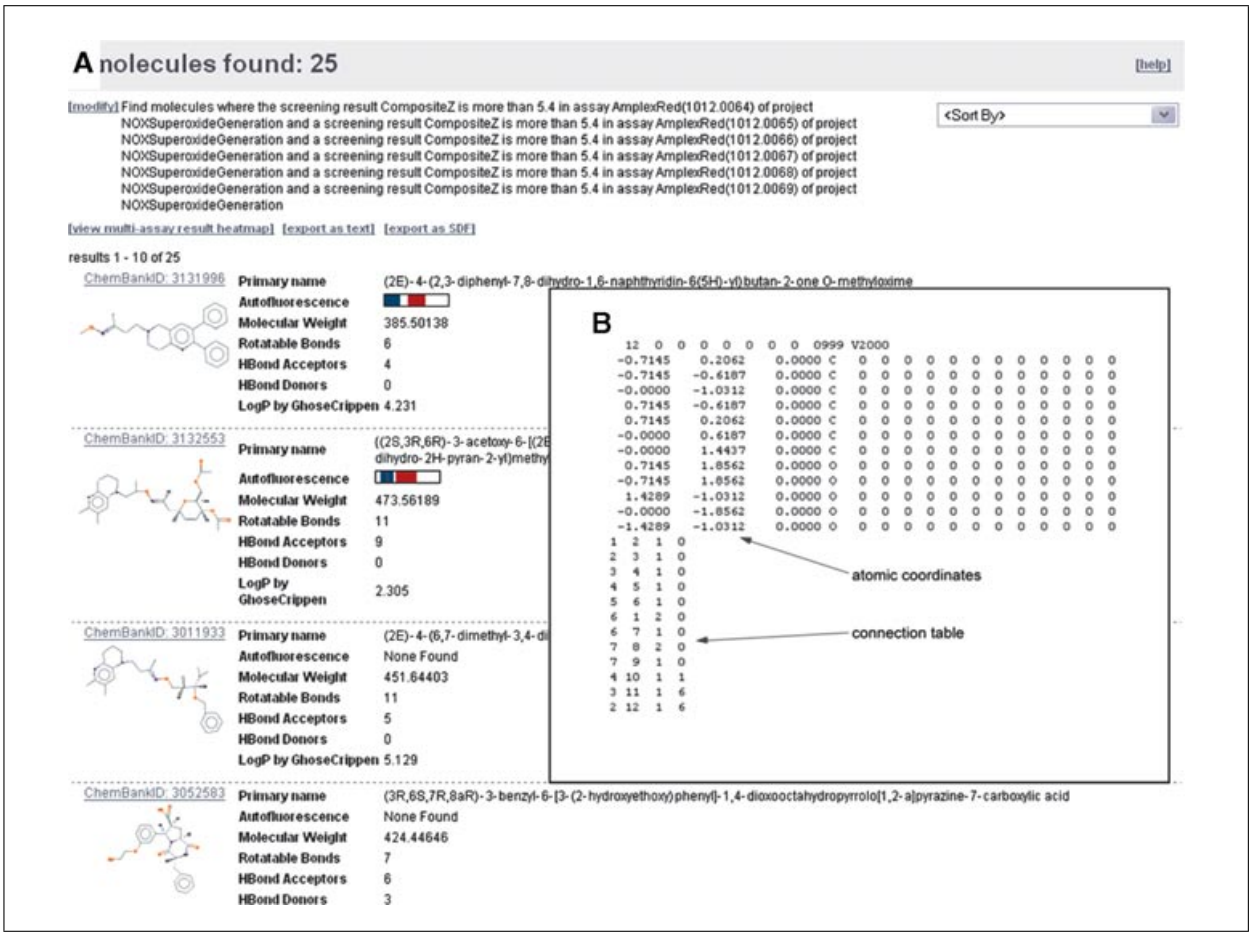
A
nolecules found: 25
[help]
[modify] Find molecules where the screening result CompositeZ is more than 5.4 in assay AmplexRed(1012.0064) of project NOXSuperoxideGeneration and a screening result CompositeZ is more than 5.4 in assay AmplexRed(1012.0065) of project NOXSuperoxideGeneration and a screening result CompositeZ is more than 5.4 in assay AmplexRed(1012.0066) of project NOXSuperoxideGeneration and a screening result CompositeZ is more than 5.4 in assay AmplexRed(1012.0067) of project NOXSuperoxideGeneration and a screening result CompositeZ is more than 5.4 in assay AmplexRed(1012.0068) of project NOXSuperoxideGeneration and a screening result CompositeZ is more than 5.4 in assay AmplexRed(1012.0069) of project NOXSuperoxideGeneration
<Sort By>
[view multi-assay result heatmap] [export as text] [export as SDF]
results 1 - 10 of 25
ChemBankID: 3131996
Primary name
(2E)-4-(2,3-diphenyl-7,8-dihydro-1,6-naphthyridin-6(5H)-yl)butan-2-one O-methyloxime
Autofluorescence
Molecular Weight 385.50138
Rotatable Bonds 6
HBond Acceptors 4
HBond Donors 0
LogP by GhoseCrippen 4.231
ChemBankID: 3132553
Primary name
Autofluorescence
Molecular Weight 473.56189
Rotatable Bonds 11
HBond Acceptors 9
HBond Donors 0
LogP by GhoseCrippen 2.305
ChemBankID: 3011933
Primary name
Autofluorescence None Found
Molecular Weight 451.64403
Rotatable Bonds 11
HBond Acceptors 5
HBond Donors 0
LogP by GhoseCrippen 5.129
ChemBankID: 3052583
Primary name (3R,6S,7R,8aR)-3-benzyl-6-[3-(2-hydroxyethoxy)phenyl]-1,4-dioxooctahydropyrrolo[1,2-a]pyrazine-7-carboxylic acid
Autofluorescence None Found
Molecular Weight 424.44646
Rotatable Bonds 7
HBond Acceptors 6
HBond Donors 3
B
atomic coordinates
connection table

Figure 14.5.7 Screenshot of `.sdf` export file output from a ChemBank download. (**A**) Search results with the "export to SDF" function highlighted. (**B**) Example of a single molecule record in `.sdf` output format, showing atomic coordinates and connection table.

26. Define a search to find all molecules that have this response pattern, repeating the procedure in steps 20 to 22 using assays "1012.0064," 1012.0065," "1012.0066," 1012.0067," "1012.0068," and "1012.0069" of the "NOXSuperoxideGeneration" project. Specify "molecules satisfying the condition" of CompositeZ scores ">5.4" for all assays.

27. Click the "search" button.

    ChemBank displays the search results in a list format.

28. Click "[export as SDF]" to generate an `.sdf` file for these molecules (see Fig. 14.5.7).

BASIC PROTOCOL 5 IDENTIFY STRUCTURALLY RELATED SMALL MOLECULES WITH KNOWN BIOLOGICAL FUNCTIONS

Imagine that compounds with a known biological function have been identified and that it is now desirable to identify structurally related compounds involved in the same biological function. This protocol describes how to use ChemBank to find molecules with the known biological function and browse their chemical structures. For a given chemical structure (the *query structure*), one can use a similarity search to find molecules with a structure similar to that of the query structure, or a substructure search to find molecules that share the query structure. It is thus possible to assemble a collection of structurally related compounds by exporting and concatenating the search results.

Necessary Resources

Hardware

A computer with a minimum of 256 Mb of RAM, connected to the Internet. A high-speed (e.g., DSL or cable modem) Internet connection is recommended, as dial-up connections will likely be exceedingly slow to load ChemBank Web pages and visualizations.

Software

A Web browser such as Internet Explorer, Firefox, or Safari is required to access ChemBank

A text editor and/or a spreadsheet program is needed to the view the contents of the downloaded `.txt` file from ChemBank

NOTE: For this example, the known biological function of the compounds of interest is their use in the treatment of asthma (Therapeutic Indication = Asthma).

Find the molecules of interest and browse their chemical structures

1. Go to the ChemBank home page (*http://chembank.broad.harvard.edu/welcome.htm*). Under Find Small Molecules, click "by function." On the "search by function" page that appears: for Ontology, select Therapeutic Indication; for Term, enter `Asthma`; also select the "Include child term matches" check box (the default). Click the "search now" button to start the search.

   Ontology terms are case-sensitive. If unsure of a term, use the Browse (magnifying glass) button to select it rather than simply typing it in.

   ChemBank displays the search in list format.

2. Examine the structures of the molecules by browsing the search results. Notice that many of the molecules include a particular four-fused-ring substructure. For this example, select ChemBankID "1000123" as having a chemical structure of interest.

Find molecules with similar chemical structures

3. Click ChemBankID "1000123" to display the Molecule Display page for that molecule.

4. Copy the SMILES string for the molecule by clicking it.

   ChemBank displays the SMILES string in a text box from which it can be copied without embedded line breaks. Copy the SMILES string from the text-box and close the pop-up window.

5. In the left-hand menu bar, under Find Small Molecules, click "by similarity." On the "Search by similarity" page, paste the SMILES string into the SMILES field. Leave the similarity metric set to Tanimoto and the similarity threshold set to 0.8. Click the "search now" button.

   A similarity threshold of 0.8 to 0.9 will return chemically intuitive results under most circumstances. A detailed description of each metric is available in the Help section of the ChemBank Web site.

   ChemBank displays the search results in list format.

Expand the search by reducing the similarity threshold from 0.8 to 0.6

6. Click "[modify]" to modify the query.

7. Click "[edit criterion]" to modify the structure criterion. The "search by similarity" page appears. Notice that the structure has been drawn on screen in the JME Molecular Editor (Ertl and Jacob, 1997).

8. On the "search by similarity" page, set the similarity threshold to `0.6` and click the "search now" button.

   ChemBank displays the search results in list format.

9. Click "[export as text]" to save the results to a text file. Rename the file `similarity_1000123.txt`.

   Only registered users are permitted to export data from ChemBank. If you logged in as "guest," you must register as a user of ChemBank to complete this step.

   A similarity search returns molecules that have the smallest number of "unshared" features when compared to the query structure. A substructure search returns molecules that share the query structure, but may have complex superstructures (extensive unshared features). For a similarity search, simplifying the query structure is likely to reduce the number of molecules found. For a substructure search, simplifying the query structure is likely to increase the number of molecules found.

Modify the query structure

10. Click "[modify]" to modify the query and "[edit criterion]" to modify the structure criteria.

11. Use the delete (DEL) function of the JME Molecular Editor (Ertl and Jacob, 1997) to simplify the chemical structure, as shown in Figure 14.5.8, panel B.

    IMPORTANT NOTE: *Due to a known software bug, when the query is modified, the chemical structure may be drawn incorrectly. To avoid this issue, set the similarity threshold to "0.6" in step 5 and skip steps 6 through 8. Alternatively, go to http://chembank.broad.harvard.edu/chemistry/search/execute.htm?id = 5050874 and begin at step 10.*

12. Click the "search now" button.

    ChemBank displays the search results in list format.

13. Click "[export as text]" to save the results to a text file. Rename the file `similarity_edited.txt`.

    Only registered users are permitted to export data from ChemBank. If you logged in as "guest," you must register as a user of ChemBank to complete this step.

    Use a substructure search to find molecules that share the edited query structure.

14. Copy the SMILES string for the edited structure as it appears in the query statement at the top of the search results page.

15. In the left-hand menu bar, under Find Small Molecules, click "by substructure." On the "search by substructure" page which appears, paste the SMILES string into the SMILES/SMARTS field and click the "search now" button.

    ChemBank displays the search results in list format.

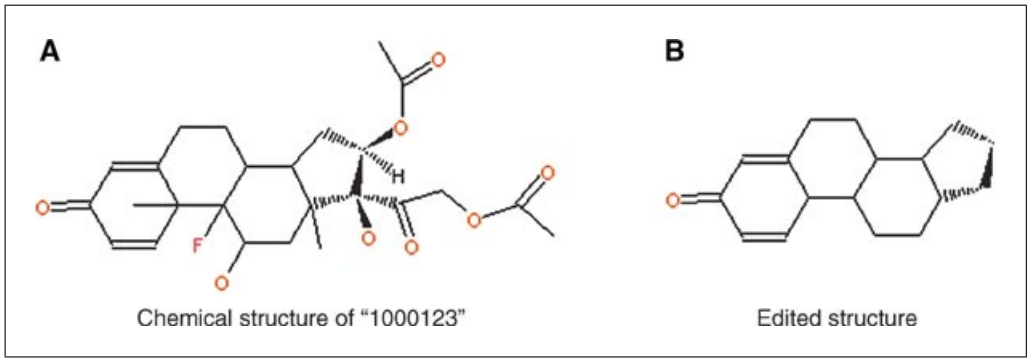

Figure 14.5.8 Structure of ChemBankID 1000123 (**A**) as shown on Molecule Display page and (**B**) simplified structure created by using the DEL function in the JME Molecular Editor (Ertl and Jacob, 1997).

16. Click "[export as text]" to save the results to a text file. Rename the file `substructure_edited.txt`.

    Only registered users are permitted to export data from ChemBank. If you logged in as "guest," you must register as a user of ChemBank to complete this step.

17. Compare the three result files.

    Sorting by ChemBankID reveals the overlap between the search results. Sorting by molecular weight reveals the higher average molecular weights of the overall larger compound structures in the substructure search result.

Determine which search was most effective in finding compounds with the known biological function of interest (Therapeutic Indication = Asthma)

18. Copy all ChemBankIDs from the first result file.

19. In the left-hand menu bar, under Find Small Molecules, click "by user list" and paste the copied values into the ChemBankIDs list box.

20. Click the "add to search" button. ChemBank displays the "molecule search builder" page.

21. From the drop-down list labeled "Select a criterion to add," select Function, and then click the "add" button.

22. On the "search by function" page: for Ontology, select Therapeutic Indication; for Term, enter Asthma; also select the "Include child term matches" check box (the default). Click the "search now" button to start the search.

23. Repeat the process for the other result files.

    After displaying details for molecule "1000123," one can find molecules with similar chemical structures by copying the SMILES string from the Molecule Display page to the "Search by similarity" page (steps 4 and 5). Alternatively, from the Molecule Display page, it is possible to find molecules with similar chemical structures by clicking "[find similar molecules]."

DOWNLOAD DATA FOR FURTHER CALCULATION USING EXTERNAL APPLICATIONS

BASIC PROTOCOL 6

For this protocol, the reader should imagine that he or she would like to download information from ChemBank for use in other applications. ChemBank provides several options for downloading data, as summarized in Table 14.5.1. In general, from a ChemBank display page, it is necessary to click "[download data]" or "[export as text]" to write the associated data to a text file.

For this example, all possible information will be downloaded for the "Aspulvinone-Upregulation" project. First, project screening data are viewed and downloaded. Next, the molecules tested in the project are viewed, with all available information about those molecules. Third, functional annotations for the molecules tested in the project are viewed and downloaded. Finally, a heatmap showing the assays in the project and the compounds tested in those assays is displayed; the heatmap data are then downloaded.

Necessary Resources

Hardware

A computer with a minimum of 256 MB of RAM, connected to the Internet. A high-speed (e.g., DSL or cable modem) Internet connection is recommended, as dial-up connections will likely be exceedingly slow to load ChemBank Web pages and visualizations.

Table 14.5.1 Downloadable Data and Fields Using the ChemBank Download/Export Functions

Object	Instructions	Downloaded data
Projects & Assays	Click "advanced assay search" Click "Search" Click "[export as text]"	ProjectID, project description, assay description, project name, project motivation, assay name, assay type, species, screener, and organization for all ChemBank projects
Project	Click "view projects" Click a project Click "[download data]"	Project name, assay name, plate, well, raw data values, background-subtracted values, CompositeZ scores, reproducibility calculations, ChemBankIDs, and SMILES strings for all compounds tested in all assays in this project
Assay	Click "advanced assay search" Click "Search" Click an assay name Click "[download data]"	Project name, assay name, plate, well, raw data values, background-subtracted values, CompositeZ scores, reproducibility calculations, ChemBankIDs, and SMILES strings for all compounds tested in this assay
Search results	Find small molecules Click "[export as text]"	ChemBankIDs and SMILES strings for all compounds on the search results page, as well as any additional information (such as Chemist, Molecule Name, or Descriptors) displayed on the search results page
Search results	Find small molecules Click "[export as SDF]"	Structure-data file (`.sdf`) for the compounds on the search results page
Heatmap	Find small molecules Click "[view multi-assay result heatmap]" Select assays Click "generate visualization" Click "[download data]"	Matrix of assay names and compounds (with SMILES strings), with CompositeZ scores for each compound in each assay (CompositeZ scores are truncated at ± 8.53)

Software

Web browser such as Internet Explorer, Firefox, or Safari is required to access ChemBank

A text editor and/or spreadsheet program is needed to view the contents of the downloaded files from ChemBank

NOTE: It is necessary to be logged into ChemBank as a registered user to download data. See Table 14.5.1 for details of downloaded data from various database objects.

View the project and download the associated screening data

1. From the menu bar on the left-hand side of the screen, click "view projects," and then select "AspulvinoneUpregulation."

   ChemBank displays the "view projects" page.

2. Click "[download data]."

   ChemBank writes the data to a tab-delimited text file and then prompts the user to open or save the file.

3. Save the file to the local hard drive. Depending on the Web browser, a prompt for directory location and filename may appear.

   The browser may need to allow popup windows for the ChemBank site to display this result properly; if a popup blocker is in use, temporarily turn it off.

   Use Microsoft Excel, a text editor, or other program of choice to view the data.

Find all molecules tested in this project

4. In the left-hand menu bar, under Find Small Molecules, click "by assay." On the "search by assay" page, check the check box for the "AspulvinoneUpregulation" project. At the bottom of the page, use the drop-down list to direct ChemBank to find "all screened molecules" (by default, ChemBank finds molecules that scored as standard hits). Click the "search now" button.

   ChemBank displays the search results in list format.

Download chemist, molecule name, and descriptor values for the compounds tested in this project, modify the query to display that information, and then download the search results

5. To add the criterion "Chemist = *", first click "[modify]" to modify the query. From the drop-down list labeled "Select a criterion to add," select Chemist and then click the "add" button. On the "search by chemist" page, input * in the text box to return the source of synthesis for every compound. Click the "search now" button.

   The asterisk (∗) substitutes as a wildcard character for any zero or more characters. In this example, entering ∗ finds every instance of the search object.

   ChemBank returns significantly more results—all compounds synthesized for all molecules tested in this project. The search results include the source of synthesis for each compound.

6. Add the criterion "Molecule Name = ∗", first click "[modify]" to modify the query. From the drop-down list labeled "Select a criterion to add," select Molecule Name and then click the "add" button. On the "search by molecule name" page which appears, input ∗ in the text box to return the molecule name for every compound. Click the "search now" button.

   The ChemBank search returns the same compounds, and the search results now include the molecule name for each compound.

7. To add the descriptor criterion "Aqueous Solubility > = –59.44," first click "[modify]" to modify the query. From the drop-down list labeled "Select a criterion to add," select Descriptor and then click the "add" button. On the "search using descriptors" page, select the first descriptor, Aqueous Solubility. To return that descriptor value for every compound, select "greater than or equal to" (> =) and input the minimum value displayed (−59.44) into the text box. Click the "search now" button.

   The ChemBank search returns fewer compounds than before, and the search results now include the Aqueous Solubility descriptor value for each compound. Fewer compounds are returned because a subset of ChemBank compounds (for example, ChemBankIDs "701" and "1484") are missing descriptor values. These compounds are removed from the search results when descriptors are added to the query.

8. Add additional descriptor criteria as desired.

9. From the search result page, click "[export as text]."

   ChemBank writes the data to a tab-delimited text file and then prompts the user to open or save the file.

10. Save the file to the local hard drive. Depending on the Web browser, there may be prompts for a directory location and file name.

    Use Microsoft Excel, a text editor, or another program of choice to view the data.

Download functional annotations for the compounds

11. Remove all previously added search criteria except the first (i.e., that the molecule was tested in any assay of project "AspulvinoneUpregulation"). To do this, click "[modify]" to modify the query, then click "[remove criterion]" next to the criterion to be removed.

12. Modify the query to add the functional annotation by choosing Function from the drop-down list labeled "select a criterion to add," and then clicking the "add" button.

13. On the "search by function" page: for Ontology, select Therapeutic Indication; for Term, use the Browse (magnifying glass) button to select the MeSH root term; also select the "Include child term matches" check box (the default). Click the "search now" button.

    ChemBank displays the compounds tested in this project that have annotations for Therapeutic Indication.

14. From the search result page, click "[export as text]" and save the results to a file.

15. On the "search by function" page: for Ontology, select Therapeutic Use; for Term, use the Browse button to select the root term ("use classification ontology"); also select the "Include child term matches" check box (the default). Click the "search now" button.

    ChemBank displays the compounds tested in this project that have annotations for Therapeutic Use.

16. From the search result page, click "[export as text]" and save the results to a file.

17. On the "search by function" page: for Ontology, select Biological Process; for Term, use the Browse button (magnifying glass) to select the root term ("biological process"); also select the "Include child term matches" check box (the default). Click the "search now" button.

    The ChemBank search returns the compounds tested in this project that have annotations for 'Biological Process.

18. From the search result page, click "[export as text]" and save the results to a file.

View a heatmap of the assays in the project and the compounds tested in those assays

19. In the left-hand menu bar, under Find Small Molecules, click "by assay." On the "search by assay" page, select the "AspulvinoneUpregulation" project. Click the "search now" button.

    By default, ChemBank finds molecules that scored as standard hits in the assays of the selected project.

    ChemBank displays the search results in list format.

20. Click "[view multi-assay result heatmap]." ChemBank displays the "select visualization features" page, which prompts the user to select the assays to display in the heatmap. Select the "AspulvinoneUpregulation" project and click the "generate visualization" button.

    ChemBank displays a heatmap (Fig. 14.5.9) of the compounds from the search result page and the assays (in the "AspulvinoneUpregulation" project) in which they were tested.

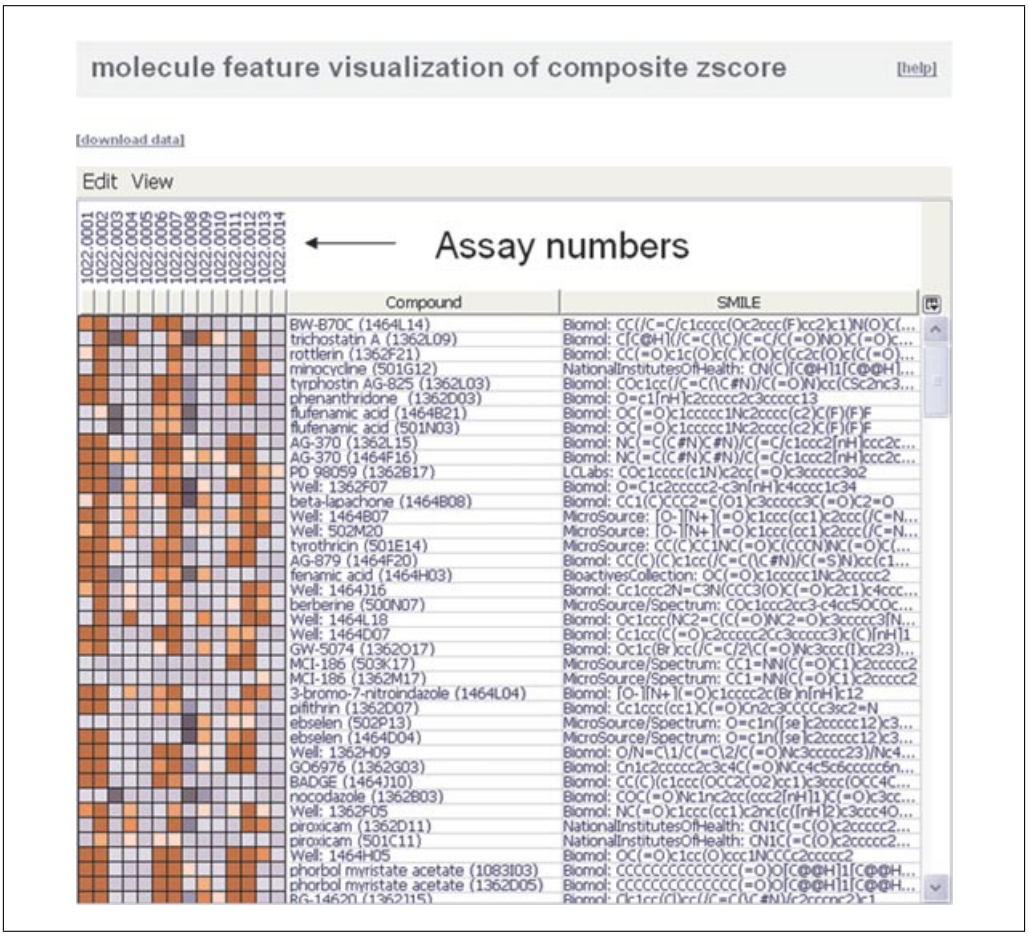

Figure 14.5.9 Screenshot of a multi-assay heatmap from ChemBank. Assays from the "AspulivnoneUpregulation" project are depicted. Compound names and SMILES for the search results are depicted in the columns to the right of the heatmap view. For the color version of this figure go to *http://www.currentprotocols.com*.

21. Click "[download data]" and save the heatmap data as a tab-delimited text file on the local hard drive. Use Microsoft Excel, a text editor, or another program of choice to view the data. CompositeZ scores in the heatmap and downloaded file are truncated at ±8.53. For actual scores, download the screening data from the View Project or View Assay page.

GUIDELINES FOR UNDERSTANDING RESULTS

The exercises in this unit have introduced some of the basic data contained within ChemBank and their potential use in a research setting. Readers have been directed to follow along with the above exercises using the "public" version of ChemBank, which contains screening data that are at least 1 year old; more recent screening data are limited to users of the screening facility at the Broad Institute and ChemBank team members, and is available at a separate URL. Data that can be viewed on ChemBank may or may not have been used in a primary publication; this information is not currently indicated on the Web site.

ChemBank is geared toward helping screeners and others contextualize and interpret high-throughput screening data by coupling its analysis to other small-molecule annotations and cheminformatics data. ChemBank is an evolving project, so new features will be added over time.

COMMENTARY

Background Information

General overview of ChemBank

ChemBank, which was created by the National Cancer Institute's Initiative for Chemical Genetics, stores information on hundreds of thousands of small molecules and hundreds of biomedically relevant assays that have been performed at the Broad Chemical Biology screening center, in collaboration with biomedical researchers worldwide. The ChemBank Web-based user interface makes it easy to retrieve this information, and the ChemBank online help (*http://chembank.broad.harvard.edu/details.htm?tag=Help*) provides descriptions of the Web pages and the data displayed (Seiler et al., 2008).

ChEBI (Brooksbank et al., 2005), DrugBank (*UNIT 14.4*; Wishart et al., 2006), PubChem (Wheeler et al., 2007), and ZINC (*UNIT 14.6*; Irwin and Shoichet, 2005), among others, are also publicly available small-molecule databases. While many of these databases are focused on discovery of novel therapeutic small molecules, ChemBank is geared not only toward chemistry and experimental results but also toward biological knowledge of small molecules from sources other than screening experiments. ChemBank stores raw screening data from the screening facility at the Broad Institute, as well as small-molecule structures from many sources. ChemBank employs a rigorous definition of screening experiments and uses a metadata-based organization for its assays and screening projects. ChemBank also allows for the visualization and analysis of both raw and normalized small-molecule assay results so that users of ChemBank can customize their views of biological results.

Molecules and compounds

In ChemBank, a ChemBankID is assigned to each unique molecule. For registration to ChemBank, standardized representations of new chemical structures are checked against the existing small-molecule collection in ChemBank and assigned an existing ChemBankID if they match an existing molecule, or a new ChemBankID if they provide a unique new structure. Individual instances of molecules are called compounds and may be salt, hydrate, or other forms for the unique molecule. Compounds are distinguished by unique Plate/Well assignments. There can be several compound samples for any given ChemBankID (molecule). Compound structures are input via a structure editor or can be entered by entering a chemistry-standard text string format, named SMILES (Weininger, 1988; Weininger and Weininger, 1989).

Projects and assays

Assays to measure the biological effects of compounds are organized into screening projects. Each project is assigned a four-digit project identifier, called a projectID. A project comprises a group of assays that all assess the same general area of biology, but may differ from each other in details of the execution protocol, the date performed, the reagents employed, or the nature of the measurement (e.g., baseline and post-incubation measurements are considered two different assays, and the calculated difference between them is a third). An assayID is the four-digit project identifier dot-separated from a four-digit assay index. Within each project, assays are uniquely numbered, usually sequentially, beginning with assay 0001. For example, an assayID might appear as the number 1012.0069, indicating the 69th assay in project 1012.

Hits and standard hits

An assay tests a collection of compounds for a single biological readout. For each tested well, ChemBank stores the associated raw value and a number of calculated values. The calculated **CompositeZ score** (Seiler et al., 2008) value is the overall measure of whether a compound scored as active in an assay. In ChemBank, the term **hit** refers to a non-zero response based on a researcher's subjective criteria. The term **standard hit** refers to a defined cutoff for the CompositeZ score and Reproducibility based on the objective criteria. For most assays, these criteria are $|\text{CompositeZ}| > 8.53$ AND $|\text{Reproducibility}| > 0.99$, where CompositeZ and Reproducibility are both calculated values stored by ChemBank. For more information about how ChemBank calculates these values, see the recent article describing ChemBank (Seiler et al., 2008).

Molecule Display page and heatmaps

In ChemBank, the Molecule Display page is the primary source of information about a molecule. It includes the name, SMILES string, descriptors, molecular structure, activity-related terms (if annotated) from the scientific literature, and all sample sources for the molecule. It also lists every screening test

instance of the molecule including the screening project, assay, plate, well, and resulting CompositeZ score. For an example, point to the URL *http://chembank.broad.harvard.edu/chemistry/viewMolecule.htm?cbid = 1347770* to display the Molecule Display page for the molecule with ChemBankID "1347770."

Heatmaps are used to visualize screening results (CompositeZ scores) for multiple compounds across multiple assays. In a multi-assay result heatmap, each row represents a compound (identified by plate/well and SMILES string) and each column represents an assay (identified by assay ID). The intersection of a row and a column represents the CompositeZ score for that compound in that assay. Dark blue represents the lowest CompositeZ scores and dark red represents the highest CompositeZ scores. Point to the URL *http://chembank.broad.harvard.edu/chemistry/featureSelection/visualize.htm?molSearchId=5046996&featureSelectId=5046999* to view a ChemBank heatmap (it takes a minute or two to gather data and display the heatmap).

Critical Parameters and Troubleshooting

It is possible to log in to ChemBank as a guest or as a registered user. Guests can try most of the scenarios; however, one must be a registered user to download any data from ChemBank (as described in Basic Protocol 6). If logged in as a guest and an attempt is made to download data, ChemBank displays an error message and offers the opportunity to register.

ChemBank supports most commonly used browsers. To check whether a browser is compatible with ChemBank, click the Browser Requirements link at the bottom of the ChemBank home page (*http://chembank.broad.harvard.edu/welcome.htm*). It may be necessary to allow popup windows from the ChemBank site, depending on the browser used. It may take ChemBank a few minutes to complete a molecule search or draw a heatmap. Any single step in a scenario should complete in less than 5 min.

One may encounter incomplete biological information or an incomplete list of names for a ChemBank page. Molecule name curation is ongoing and not all molecules have gone through curation, and therefore may be missing commonly known names. Additionally, one may encounter more than one molecule with the same name but slightly different structures. Structures in ChemBank are often loaded from vendor-supplied files and are not independently verified by the ChemBank team. Molecular structure verification and merging of database records is planned for future ChemBank enhancements.

Many of the biological annotations on ChemBank pages are taken from the primary literature. PubMed IDs, when available, appear as a hyperlink to the right of the biological annotation. Additionally, users cannot currently search biological annotations by GO molecular function terms (*UNIT 7.2*). GO terms may also be out of date, as ChemBank updates its local version of GO only periodically. GO terms are planned to be fully searchable, regularly updated, and hyperlinked in future ChemBank releases.

The following are a few tips for using ChemBank heatmaps:

1. Select Color Scheme Legend from the View menu to display a legend for the colors. The colors in a heatmap always range from dark blue to dark red; however, the CompositeZ scores represented by those colors vary from one heatmap to another. Dark blue represents the lowest CompositeZ score in the heatmap and dark red the highest CompositeZ score in the heatmap.

2. Hover the cursor over a cell to display the associated compound name, assay ID, and CompositeZ score.

3. Double-click an assayID (column header) to display its details. Double-click a compound name (right side of the heatmap) to display its Molecule Display page.

4. Heatmaps truncate CompositeZ scores at ± 8.53 (the ChemBank cutoff for a standard hit). To see the precise CompositeZ scores, display the Molecule Display page for a compound.

5. For a more readable display, sort the heatmap in one of three ways: click the box below an assay ID (column header) to sort compounds based on their CompositeZ scores in that assay; click the Compound column heading (right side of the heatmap) to sort compounds by name and plate number; or click the SMILES column heading (right side of the heatmap) to sort compounds by SMILES string. Sorting by SMILES string is a crude way of grouping compounds by structure similarity.

To report problems, click the Report Problem link at the bottom of any ChemBank page. To submit questions not answered by this unit or the online help, contact the ChemBank team by e-mailing *chembank@broad.harvard.edu*.

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Using ZINC to Acquire a Virtual Screening Library

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ABSTRACT

The ZINC database of commercially available compounds contains biologically relevant representations of purchasable compounds in ready-to-screen formats. ZINC uses catalogs from over 50 compound vendors, changing from time to time to reflect newly available compounds and depleted stock. ZINC is available in subsets that reflect current opinion in the field such as "fragment-like" and "lead-like," and has a facility to create additional small ad hoc subsets. ZINC has a search facility, as well as a service to process molecules that are not in ZINC by uploading them to a server. *Curr. Protoc. Bioinform.* 22:14.6.1-14.6.23. © 2008 by John Wiley & Sons, Inc.

Keywords: virtual screening • molecular docking • ligand discovery • small molecule libraries

INTRODUCTION

The ZINC database of commercially available compounds for virtual screening contains biologically relevant representations of purchasable compounds for ligand discovery. ZINC aggregates catalogs from chemical suppliers and processes them into biologically relevant forms, reformats them into popular file formats, and distributes them in a variety of subsets. The ZINC Web site offers a search capability, custom subset preparation, and a facility for processing molecules that are uploaded to the site.

ZINC is based on a processing pipeline that converts two-dimensional vendor catalog entries into biologically relevant forms. ZINC contains protonated, deprotonated, and tautomeric forms of molecules classified over three pH ranges. Whereas ZINC was originally designed with structural (3-D) ligand discovery methods like molecular docking and virtual screening in mind, it has also proven useful for topological (2-D) efforts, including similarity searching, scaffold hopping, clustering, and classification approaches. Thus medicinal chemists, drug designers, and structural biologists as well as bioinformaticians and data miners are all potential users of ZINC. ZINC focuses on commercially available compounds to shorten the hypothesis-test cycle in early-stage ligand discovery. It also includes annotated ligands from PubChem, many of which are in turn linked to annotated databases or other sources of information. ZINC utilizes chemical software packages and resources including CACTVS (Ihlenfeldt et al., 1992; *http://xemistry.com*), CORINA (Gasteiger et al., 1990; *http://www.molecular-networks.com*), OEChem and Omega (OpenEye Scientific Software; *http://www.eyesopen.com*), mi-tools (Molinspiration; *http://www.molinspiration.com*), AMSOL (Chris Cramer and Don Truhlar; *http://amsol.chem.umn.edu/*), and ligprep (Schrödinger Inc., *http://www.schrodinger.com*).

The five protocols in this unit describe the most common procedures that researchers will use to acquire computer representations of purchasable compounds from ZINC. If you want to screen for novel ligands without the bias of a chemical starting point, we recommend starting with Basic Protocol 1. Even if you already have one or more actives for your project, you may still want to acquire one or more of the general purpose screening libraries available via Basic Protocol 1. We recommend either the

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"fragment-like" or "lead-like" subsets of ZINC, which best represent current thinking in the field. For 3-D applications like docking, use either mol2 or SDF format. For 2-D methods like scaffold hopping via molecular similarity metrics, you may want to use SMILES. Once downloaded to your local disk, use your screening application to identify compounds to acquire and test. If you have a special pricing deal with a particular vendor or your screening center has purchased compounds from a single vendor, for example, you will want to use Basic Protocol 2 to acquire a vendor-specific subset of ZINC. If you already have actives and you prefer to screen only a small set of related molecules, start with Basic Protocol 3 to find molecules that match your criteria, and download up to 500 of them. Basic Protocol 3 is also useful for browsing ZINC to see what is inside. A number of protocols that are alternatives to Basic Protocol 3 illustrate a range of supported queries. If you require a larger subset, up to 10,000 molecules, use Basic Protocol 4 to create one and acquire it. If you need a custom subset with >10,000 molecules, please contact *support@docking.org* to request it. Finally, if the molecules that you wish to screen do not exist in ZINC, you will want to use Basic Protocol 5, which processes molecules you upload using the standard ZINC processing pipeline.

The ZINC search service, detailed in Basic Protocol 3, offers a variety of options for searching ligands in ZINC based on substructure, physicochemical properties, catalog information, or a combination of these constraints. ZINC can be useful for finding similar or dissimilar compounds, using either SMARTS or SMILES (see Daylight Theory Manual; *http://daylight.com*). The results of a ZINC search point directly to compound vendors, to facilitate compound acquisition. Pointers to PubChem and other public annotated databases are also included where available, as a source of annotations about the molecule. This unit describes the use of ZINC version 7 for Basic Protocols 1, 2, and 3. These protocols are nearly the same for ZINC version 8—the release of which is expected soon—except the second step involves using a pull-down menu in ZINC 8 instead of simply clicking on a link. Basic Protocols 4 and 5 are only supported in ZINC 8, which is accessible at *http://zinc8.docking.org/*.

BASIC PROTOCOL 1

DOWNLOAD A PROPERTY-FILTERED DATABASE SUBSET FOR VIRTUAL SCREENING

This protocol describes how to acquire a property-filtered database subset of small molecules in ready-to-dock formats for general purpose virtual screening of commercially available chemical space. The two most popular of these are the "lead-like" and "fragment-like" subsets. The subsets are available in three popular formats over three pH ranges. Large files are split into slices for more reliable downloading. At the end of this protocol, you will have a database of small molecules on your disk that are ready to screen.

Necessary Resources

Hardware

A modern computer with an Internet connection. Some files are very large, so 100 GB or more of free space may be required to store the uncompressed files in mol2 format.

Software

A Unix-like environment, such as Unix, Linux, Mac OS X, or Cygwin. See Support Protocol of *UNIT 9.6* for installation of Cygwin. Other operating systems may require minor changes.

If using Windows, wget is needed; this is available from SourceForge (*http://sourceforge.net/*) or the Web site *http://wget.docking.org/*. It may be easier to download ZINC files to a Unix-like machine and move the files to Windows.

A modern browser, such as Firefox 1.5 or later, Opera 9 or later, or Internet Explorer 7 or later (Internet Explorer 6 will work, but is not advised), with the Java Runtime Environment (JRE). JRE is available from *http://java.sun.com/jre/* if not already installed.

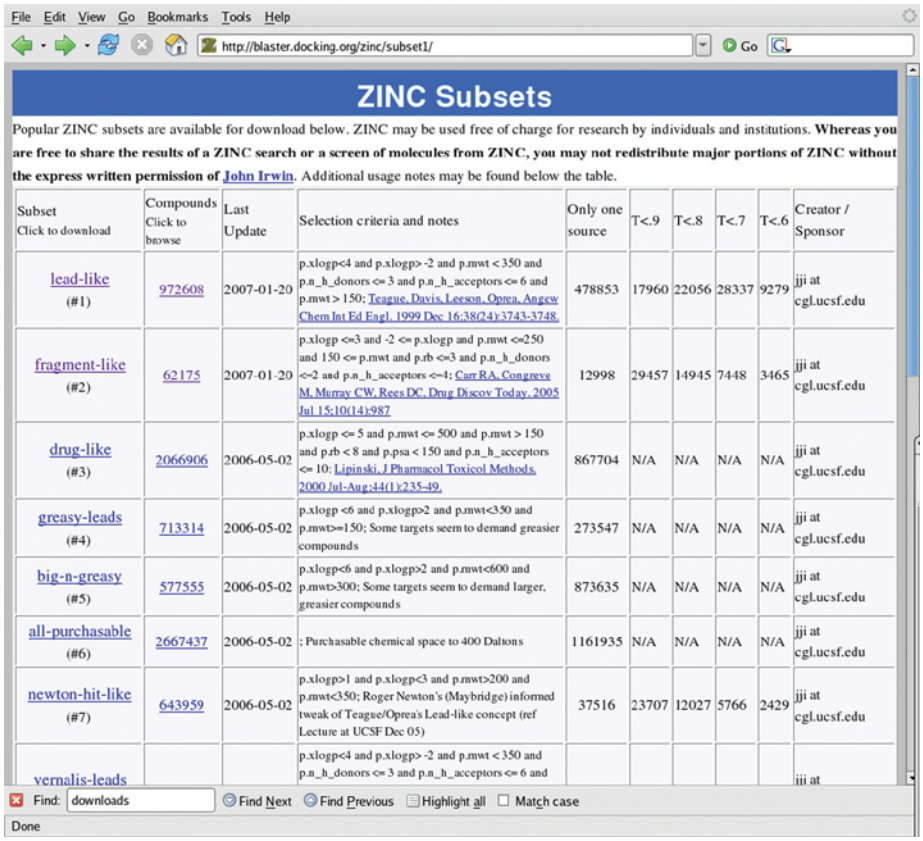
File Edit View Go Bookmarks Tools Help
http://blaster.docking.org/zinc/subset1/
Go
ZINC Subsets
Popular ZINC subsets are available for download below. ZINC may be used free of charge for research by individuals and institutions. Whereas you are free to share the results of a ZINC search or a screen of molecules from ZINC, you may not redistribute major portions of ZINC without the express written permission of John Irwin. Additional usage notes may be found below the table.
Subset Click to download | Compounds Click to browse | Last Update | Selection criteria and notes | Only one source | T<.9 | T<.8 | T<.7 | T<.6 | Creator / Sponsor
lead-like (#1) | 972608 | 2007-01-20 | p.xlogp<4 and p.xlogp> -2 and p.mwt < 350 and p.n_h_donors <= 3 and p.n_h_acceptors <= 6 and p.mwt > 150; Teague, Davis, Leeson, Oprea, Angew Chem Int Ed Engl. 1999 Dec 16;38(24):3743-3748. | 478853 | 17960 | 22056 | 28337 | 9279 | jji at cgl.ucsf.edu
fragment-like (#2) | 62175 | 2007-01-20 | p.xlogp <=3 and -2 <= p.xlogp and p.mwt <=250 and 150 <= p.mwt and p.rb <=3 and p.n_h_donors <=2 and p.n_h_acceptors <=4; Carr RA, Congreve M, Murray CW, Rees DC, Drug Discov Today. 2005 Jul 15;10(14):987 | 12998 | 29457 | 14945 | 7448 | 3465 | jji at cgl.ucsf.edu
drug-like (#3) | 2066906 | 2006-05-02 | p.xlogp <= 5 and p.mwt <= 500 and p.mwt > 150 and p.rb < 8 and p.psa < 150 and p.n_h_acceptors <= 10; Lipinski, J Pharmacol Toxicol Methods. 2000 Jul-Aug;44(1):235-49. | 867704 | N/A | N/A | N/A | N/A | jji at cgl.ucsf.edu
greasy-leads (#4) | 713314 | 2006-05-02 | p.xlogp <6 and p.xlogp>2 and p.mwt<350 and p.mwt>=150; Some targets seem to demand greasier compounds | 273547 | N/A | N/A | N/A | N/A | jji at cgl.ucsf.edu
big-n-greasy (#5) | 577555 | 2006-05-02 | p.xlogp<6 and p.xlogp>2 and p.mwt<600 and p.mwt>300; Some targets seem to demand larger, greasier compounds | 873635 | N/A | N/A | N/A | N/A | jji at cgl.ucsf.edu
all-purchasable (#6) | 2667437 | 2006-05-02 | ; Purchasable chemical space to 400 Daltons | 1161935 | N/A | N/A | N/A | N/A | jji at cgl.ucsf.edu
newton-hit-like (#7) | 643959 | 2006-05-02 | p.xlogp>1 and p.xlogp<3 and p.mwt>200 and p.mwt<350; Roger Newton's (Maybridge) informed tweak of Teague/Oprea's Lead-like concept (ref Lecture at UCSF Dec 05) | 37516 | 23707 | 12027 | 5766 | 2429 | jji at cgl.ucsf.edu
vernalis-leads | | | p.xlogp<4 and p.xlogp> -2 and p.mwt < 350 and p.n_h_donors <= 3 and p.n_h_acceptors <= 6 and | | | | | | jji at
Find: downloads
Find Next
Find Previous
Highlight all
Match case
Done

Figure 14.6.1 The ZINC Property-Filtered Subsets page.

To download the "lead-like" subset in mol2 format

1. Point the browser to *http://zinc.docking.org*.
2. Click on the Property-filtered Subsets link to see available property-based database subsets.

   You will see a table of available subsets (Fig. 14.6.1). In addition to "lead-like," other subsets are available, such as "fragment-like" and "all-purchasable." For general-purpose screening, the "lead-like" and "fragment-like" subsets are the most popular and are representative of current opinion in the field. The table of database subsets (Fig. 14.6.1) lists the name of the subset, number of molecules in each subset, date of the last update, criteria used to select molecules, and number of compounds available from a single source. The table provides a thumbnail sketch of how "chemically diverse" each subset is, expressed as the number of representatives required to represent the subset at various Tanimoto (T) similarity levels. Thus, for the lead-like subset, all 972,608 compounds are at least 60% similar to at least one of 9279 representatives and at least 70% similar to at least one of 28,337 representatives. The sponsor of the subset, to whom any correspondence should be addressed, is the final item.

3. Click on "lead-like," in the first row, left-most column, to go to the lead-like download page.

   The "lead-like" download page (Fig. 14.6.2) contains detailed information about the subset organized into four sections: (1) General Information, (2) Property Distributions, (3) Clustering and Diversity, and (4) Downloads.

   General Information lists the following information: subset name, subset number, number of entries, selection criteria, when and by whom the subset was created, and additional filtering constraints.

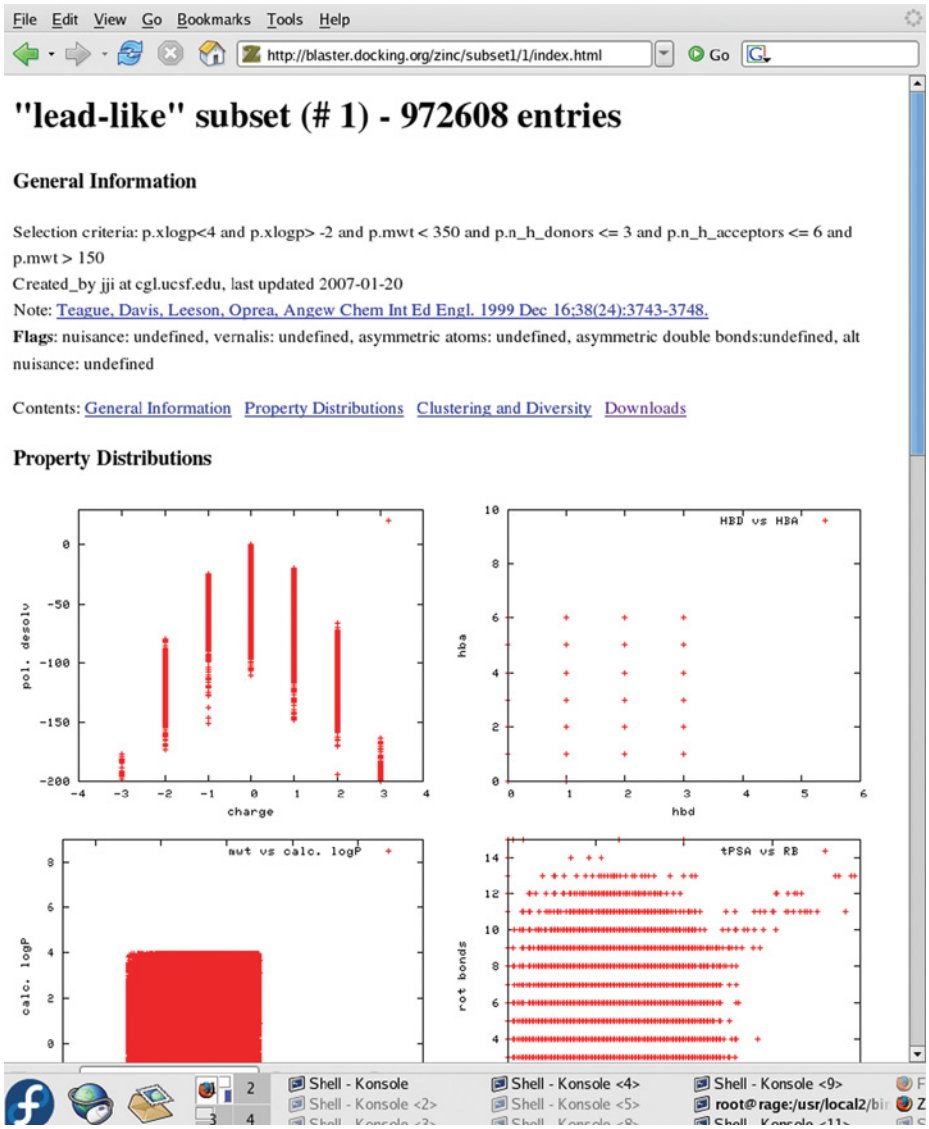
File Edit View Go Bookmarks Tools Help
http://blaster.docking.org/zinc/subset1/1/index.html
Go
"lead-like" subset (# 1) - 972608 entries
General Information
Selection criteria: p.xlogp<4 and p.xlogp> -2 and p.mwt < 350 and p.n_h_donors <= 3 and p.n_h_acceptors <= 6 and p.mwt > 150
Created_by jji at cgl.ucsf.edu, last updated 2007-01-20
Note: Teague, Davis, Leeson, Oprea, Angew Chem Int Ed Engl. 1999 Dec 16;38(24):3743-3748.
Flags: nuisance: undefined, vernalis: undefined, asymmetric atoms: undefined, asymmetric double bonds:undefined, alt nuisance: undefined
Contents: General Information Property Distributions Clustering and Diversity Downloads
Property Distributions
pol. desolv
charge
HBD vs HBA
hba
hbd
mwt vs calc. logP
calc. logP
tPSA vs RB
rot bonds
Shell - Konsole
Shell - Konsole <2>
Shell - Konsole <4>
Shell - Konsole <5>
Shell - Konsole <9>
root@rage:/usr/local2/bi

Figure 14.6.2 The ZINC database lead-like subset download page.

Under Property Distribution, scatter plots offer an immediate if limited means of evaluating the distribution of molecular properties in the subset.

The representative clusters listed in the Clustering and Diversity section offer four levels of selected representatives that cover the same range of chemical diversity as the entire subset.

The Downloads section contains a table of links with which to download molecules in various formats, over various pH ranges, as well as purchasing information, molecular properties, and compounds with a sole supplier.

4. Click on the Downloads link (near the top of the page under the General Information section) to go to the download section of the page at the bottom (Fig. 14.6.3), or simply scroll to the bottom of the page where the Downloads section and download table are located.

5. Click on Usual in the right-most column of the "mol2" row of the download table.

   This downloads a C-shell script (`usual.mol2.csh`*) that will be used to acquire the mol2 format molecular structure files for molecular representations at or near*

physiological pH. The Usual subset includes single representations of each molecule plus any additional protonated or tautomeric forms near physiological pH. Other choices include Single, to download only a single representation of each molecule (at pH 7), Metals, to download Usual forms plus additional high-pH forms, such as deprotonated sulfonamides and thiolates, and All, which includes additional low-pH forms such as protonated anilines, for example.

6. Invoke the C-shell to run the script:

```
unix> csh usual.mol2.csh
```

This runs the `usual.mol2.csh` *script to download the database in compressed mol2 format. This script uses wget to download all files automatically with a single command. If you are on Linux, Mac OS X, Cygwin, or another Unix-like platform, this script will work as long as wget is available. If you are on Windows, you will need wget, available from SourceForge or http://wget.docking.org as described above. It is also possible to download each database slice individually by clicking on individual slices in the download table. Whereas downloading files individually is a viable strategy for smaller subsets, such as the "fragment-like" subset, it becomes impractical for larger database subsets with many slices. Downloading the "lead-like" subset can take hours, depending on the speed of your Internet connection. To download SDF instead of mol2, follow the same procedure as above (i.e., clicking Usual in the rightmost column of the SDF row in the download table; the script downloaded will then be* `usual.sdf.csh`*). SDF and mol2 format files contain largely the same information, so normally you will only want one or the other. SMILES-format files (see top row of download table) are much smaller, so you download SMILES directly, rather than scripts, to download them. Thus there are no*

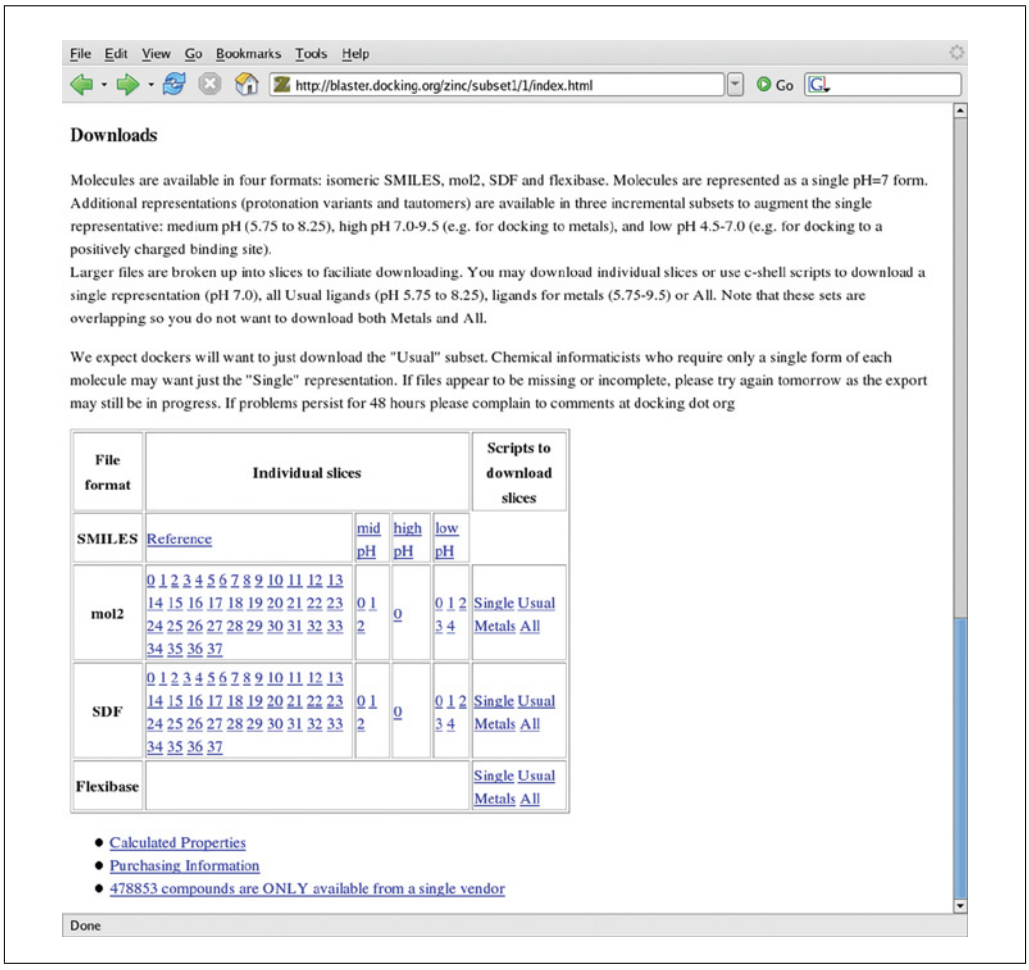
File Edit View Go Bookmarks Tools Help
http://blaster.docking.org/zinc/subset1/1/index.html
Go
Downloads
Molecules are available in four formats: isomeric SMILES, mol2, SDF and flexibase. Molecules are represented as a single pH=7 form. Additional representations (protonation variants and tautomers) are available in three incremental subsets to augment the single representative: medium pH (5.75 to 8.25), high pH 7.0-9.5 (e.g. for docking to metals), and low pH 4.5-7.0 (e.g. for docking to a positively charged binding site).
Larger files are broken up into slices to faciliate downloading. You may download individual slices or use c-shell scripts to download a single representation (pH 7.0), all Usual ligands (pH 5.75 to 8.25), ligands for metals (5.75-9.5) or All. Note that these sets are overlapping so you do not want to download both Metals and All.
We expect dockers will want to just download the "Usual" subset. Chemical informaticists who require only a single form of each molecule may want just the "Single" representation. If files appear to be missing or incomplete, please try again tomorrow as the export may still be in progress. If problems persist for 48 hours please complain to comments at docking dot org
File format
Individual slices
Scripts to download slices
SMILES
Reference
mid pH
high pH
low pH
mol2
0 1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 23 24 25 26 27 28 29 30 31 32 33 34 35 36 37
0 1 2
0
0 1 2 3 4
Single Usual Metals All
SDF
0 1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 23 24 25 26 27 28 29 30 31 32 33 34 35 36 37
0 1 2
0
0 1 2 3 4
Single Usual Metals All
Flexibase
Single Usual Metals All
Calculated Properties
Purchasing Information
478853 compounds are ONLY available from a single vendor
Done

Figure 14.6.3 Bottom of the "lead-like" download page, featuring the download table.

C-shell scripts to download SMILES. Flexibase files (see bottom row of download table) are only read by DOCK3.5.54, a version of UCSF DOCK, and may not be available for all subsets. If you are docking to a metallo-enzyme, you may want to consider using the Metals option instead of Usual to download additional high-pH representations. If you are doing substructure searches, clustering, or pattern matching, you may prefer to use the Single option instead, which offers a single representation of each molecule at pH 7 only.

If your docking program does not read gzip-compressed files, then you will need to uncompress the files before using them, using gunzip. Warning, these files can be large!

7. When downloading of the database in mol2 format, as described in step 6, is complete, execute:

```
unix> gunzip *.mol2.gz; # optional
```

At this point you have a database on your disk ready for screening. This protocol works equally well for all the subsets on the subset download page. Some users may be interested in optional additional information about this subset, as described below.

To view and acquire additional information about the "lead-like" subset

8. Click on the Reference link in the SMILES row of the download table at the bottom of the "lead-like" download page (Fig. 14.6.3).

This downloads a single representation of each molecule in the subset as SMILES, which can be useful for local similarity searching, cluster analysis, and scaffold hopping,

9. Go to the Clustering and Diversity section of the download page (Fig. 14.6.4) by scrolling, or by clicking on the Clustering and Diversity link at the top of the page.

10. Click on the "80%" link.

This downloads cluster representatives at the Tanimoto 80% level as SMILES. All 972,608 lead-like compounds are within Tanimoto 80% of one of these 83,331 representatives. Cluster representatives may be useful for fast, approximate screens, or to save time. For example, you might choose to screen only some representatives of the subset to get an impression of what might be found via a larger database screen. To download cluster

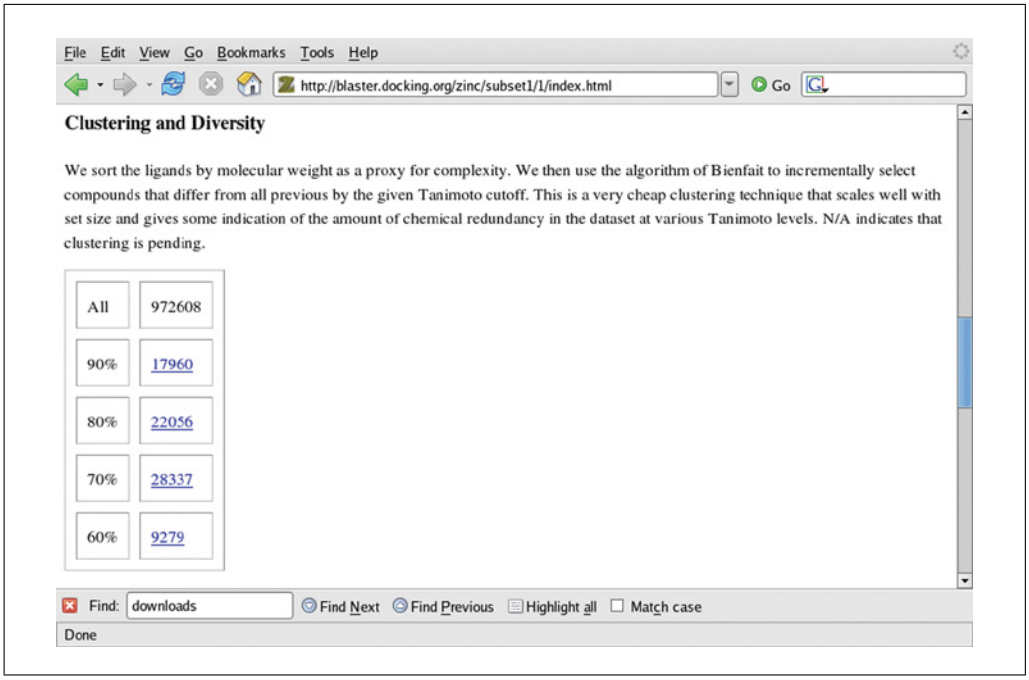
File Edit View Go Bookmarks Tools Help
http://blaster.docking.org/zinc/subset1/1/index.html
Go
Clustering and Diversity
We sort the ligands by molecular weight as a proxy for complexity. We then use the algorithm of Bienfait to incrementally select compounds that differ from all previous by the given Tanimoto cutoff. This is a very cheap clustering technique that scales well with set size and gives some indication of the amount of chemical redundancy in the dataset at various Tanimoto levels. N/A indicates that clustering is pending.
All 972608
90% 17960
80% 22056
70% 28337
60% 9279
Find: downloads
Find Next
Find Previous
Highlight all
Match case
Done

Figure 14.6.4 Clustering and Diversity section of the "lead-like" download page.

representatives, there are four sets of representatives at the 60%, 70%, 80%, and 90% Tanimoto levels, offering a range of set sizes to choose from.

11. Click on the Purchasing Information link at the bottom of the download page (Fig. 14.6.3).

    If you intend to purchase compounds to test your predictions, you may wish to download purchasing information. This file is tab-delimited text containing ZINC ID, catalog number, supplier name, and contact information for each compound. If there are <64,000 rows (e.g., for the "fragment-like" subset), then this file may be opened in Excel or OpenOffice. If there are >64,000 rows, right mouse click to save file, and use a text editor such as vim or emacs.

12. Click on the Calculated Properties link at the bottom of the download page (Fig. 14.6.3).

    This downloads a table of calculated properties for each molecule. The columns are: ZINC ID, molecular weight, calculated LogP, apolar desolvation energy (kcal/mol), polar desolvation energy (kcal/mol), number of hydrogen bond donors, number of hydrogen bond acceptors, parametric polar surface area, net molecular charge, number of rotatable bonds, and the SMILES representation. If there are <64,000 rows, then this file may be opened in Excel or OpenOffice. If there are >64,000 rows, right mouse click to save file, and use a text editor such as vim or emacs.

13. Click on the last link on the "lead-like" download page to acquire a list of compounds that can only be purchased from a single supplier (Fig. 14.6.3).

    This downloads a table of compounds that are only available from a single supplier (as far as we know). Again, due to limitations of spreadsheets, right click to save files with >64,000 rows.

14. Click the Back button in your browser to return to the list of available property-filtered subsets (i.e., the page shown in Fig. 14.6.1).

15. Click on the number in the Compounds column in the row corresponding to the subset of interest on the Property-filtered Subset page to browse the subset online (Fig. 14.6.1).

    This allows you to browse the content of available subsets online. You might want to do this with each subset before you download it, to check whether you are interested in it. Each page displays 500 molecules at a time. Thus for large subsets, you are only seeing a very small fraction of the subset.

    When you have finished this protocol, you will have a database of commercially available compounds that is ready to dock (see UNIT 8.12), screen, classify, or otherwise interpret. You may also have additional information if you performed any of the steps 10 to 15. When downloading so many large files, errors in transmission may occur.

16. You may check that you successfully acquired the entire database subset by counting the number of unique ZINC ID numbers that are included in the files you downloaded, as follows:

```
unix> grep ZINC *.mol2 | sort -u | wc -l
```

17. If you have kept the files compressed, then use:

```
unix> grep ZINC *.mol2.gz | sort -u | wc -l
```

    The result of this command should be compared with the number of molecules in the subset, listed at the top of the download page (Fig. 14.6.2). If the numbers differ by >1%, then compare the number of subset slices with the number in the `usual.mol2.csh` *script and with the number of slices listed on the download page. Incomplete, missing, or damaged files may be re-downloaded individually by clicking on the slice number in the download table at the bottom of the download Web page (Fig. 14.6.3).*

DOWNLOAD A VENDOR DATABASE SUBSET FOR VIRTUAL SCREENING

Use this protocol to acquire biologically relevant representations of molecules for screening from a single supplier. You might want to do this because you have a special pricing deal with a particular vendor, because you are involved in a collaboration that favors a particular vendor, or because you may have already purchased a vendor's collection for HTS and you wish to complement that effort with virtual screening. Whatever your reason, this is the protocol to use. PubChem, which is not a vendor but whose molecules are linked to the chemical and biological literature via the NIH Entrez system, may be useful for identifying ligands with biological annotations. Similarly, the Molecular Libraries Screening Center Network screening collection (MLSCN), which is the screening library used at nine extramural NIH-funded centers and the National Chemical Genomics Center, may be of interest because biological activity data for its compounds are available in the PubChem Assay database.

Necessary Resources

Hardware

A modern computer with an Internet connection. Some files are very large, so 100 GB or more of free space may be required to store the uncompressed files in mol2 format.

Software

A Unix-like environment, such as Unix, Linux, Mac OS X, or Cygwin. See Support Protocol of *UNIT 9.6* for installation of Cygwin. Other operating systems may require minor changes.

If using Windows, wget is needed; this is available from SourceForge (*http://sourceforge.net/*) or the Web site *http://wget.docking.org/*. It may be easier to download ZINC files to a Unix-like machine and move the files to Windows.

A modern browser, such as Firefox 1.5 or later, Opera 9 or later, or Internet Explorer 7 or later (Internet Explorer 6 will work, barely, but is not advised), with the Java Runtime Environment (JRE). JRE is available from *http://java.sun.com/jre/* if not already installed.

To download the Enamine vendor subset in mol2 format

1. Point the browser to *http://zinc.docking.org*.
2. Click on the Vendors link to see available vendor database subsets, sorted alphabetically (Fig. 14.6.5).
3. For this example, scroll down to the Enamine subset. You could equally well use any other vendor.

   About 50 vendor subsets are available, one for each vendor. This protocol works equally well for all vendors. The table of vendor subsets (Fig. 14.6.5) contains one row for each vendor with the following information and features organized in five cells. First, the vendor logo may be clicked to browse the subset online. The second cell contains supplier information including name, Web site, e-mail, phone, and fax numbers. The third cell contains the number of source catalog entries and the catalog version used. The fourth cell contains information about this subset in ZINC: number of molecules loaded, number filtered out, number for which this vendor is the sole source, and number of compounds in previous catalogs that are no longer in stock (termed "depleted"). Diversity information comes last in the form of four sets of cluster representatives at the 60%, 70%, 80%, and 90% Tanimoto levels as was done in Basic Protocol 1. For example, for Enamine, all 876,780 compounds are at least 90% similar to at least one of the 241,664 90% cluster representatives, reflecting a >3-fold reduction in the size of the set at the Tanimoto 90% level.

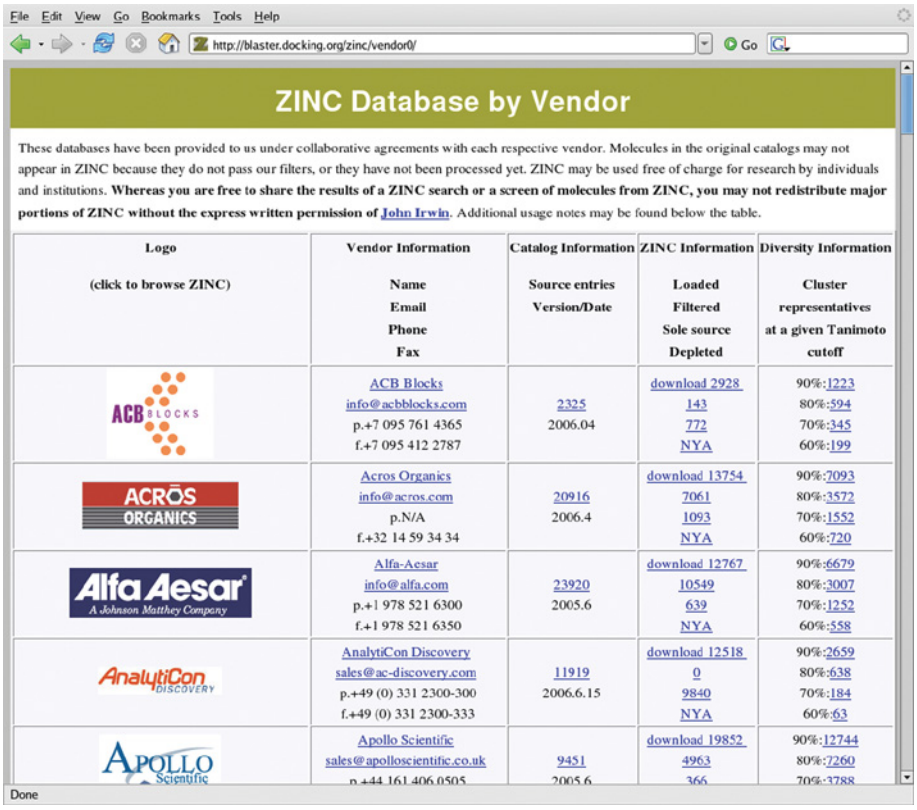
File Edit View Go Bookmarks Tools Help
http://blaster.docking.org/zinc/vendor0/
Go
ZINC Database by Vendor
These databases have been provided to us under collaborative agreements with each respective vendor. Molecules in the original catalogs may not appear in ZINC because they do not pass our filters, or they have not been processed yet. ZINC may be used free of charge for research by individuals and institutions. Whereas you are free to share the results of a ZINC search or a screen of molecules from ZINC, you may not redistribute major portions of ZINC without the express written permission of John Irwin. Additional usage notes may be found below the table.
Logo (click to browse ZINC)
Vendor Information Name Email Phone Fax
Catalog Information Source entries Version/Date
ZINC Information Loaded Filtered Sole source Depleted
Diversity Information Cluster representatives at a given Tanimoto cutoff
ACB Blocks info@acbblocks.com p.+7 095 761 4365 f.+7 095 412 2787
2325 2006.04
download 2928 143 772 NYA
90%:1223 80%:594 70%:345 60%:199
Acros Organics info@acros.com p.N/A f.+32 14 59 34 34
20916 2006.4
download 13754 7061 1093 NYA
90%:7093 80%:3572 70%:1552 60%:720
Alfa-Aesar info@alfa.com p.+1 978 521 6300 f.+1 978 521 6350
23920 2005.6
download 12767 10549 639 NYA
90%:6679 80%:3007 70%:1252 60%:558
AnalytiCon Discovery sales@ac-discovery.com p.+49 (0) 331 2300-300 f.+49 (0) 331 2300-333
11919 2006.6.15
download 12518 0 9840 NYA
90%:2659 80%:638 70%:184 60%:63
Apollo Scientific sales@apolloscientific.co.uk
9451
download 19852 4963 366
90%:12744 80%:7260
Done

Figure 14.6.5 ZINC vendor subsets.

4. Click on the number 876780 in the fourth column to go to the Enamine download page (Fig. 14.6.6).

   The Enamine download page (Fig. 14.6.6) contains detailed information about the subset organized into four sections, analogous to the organization of the "lead-like" download page in Basic Protocol 1, as follows: (1) General Information, (2) Property Distributions, (3) Clustering and Diversity, and (4) Downloads.

   General Information lists the following information: subset name, subset number, number of entries, selection criteria, when and by whom the subset was created, and additional filtering constraints.

   Under Property Distribution, scatter plots offer an immediate if limited means of evaluating the distribution of molecular properties in the subset.

   The representative clusters listed in the Clustering and Diversity section offer four levels of selected representatives that cover the same range of chemical diversity as the entire subset.

   The Downloads section contains a table of links with which to download molecules in various formats, over various pH ranges, as well as purchasing information, molecular properties, and compounds for which this vendor is the sole supplier.

5. Click on the Downloads link (near the top of the page under the General Information section) to go to the download section of the page near the bottom, or simply scroll to the bottom of the page to see the available files to download (refer to Fig. 14.6.7).

6. In the table of downloadable files, click on Usual in the "sdf" row.

   This downloads a csh script that will be used to acquire the molecular structure files in SDF format.

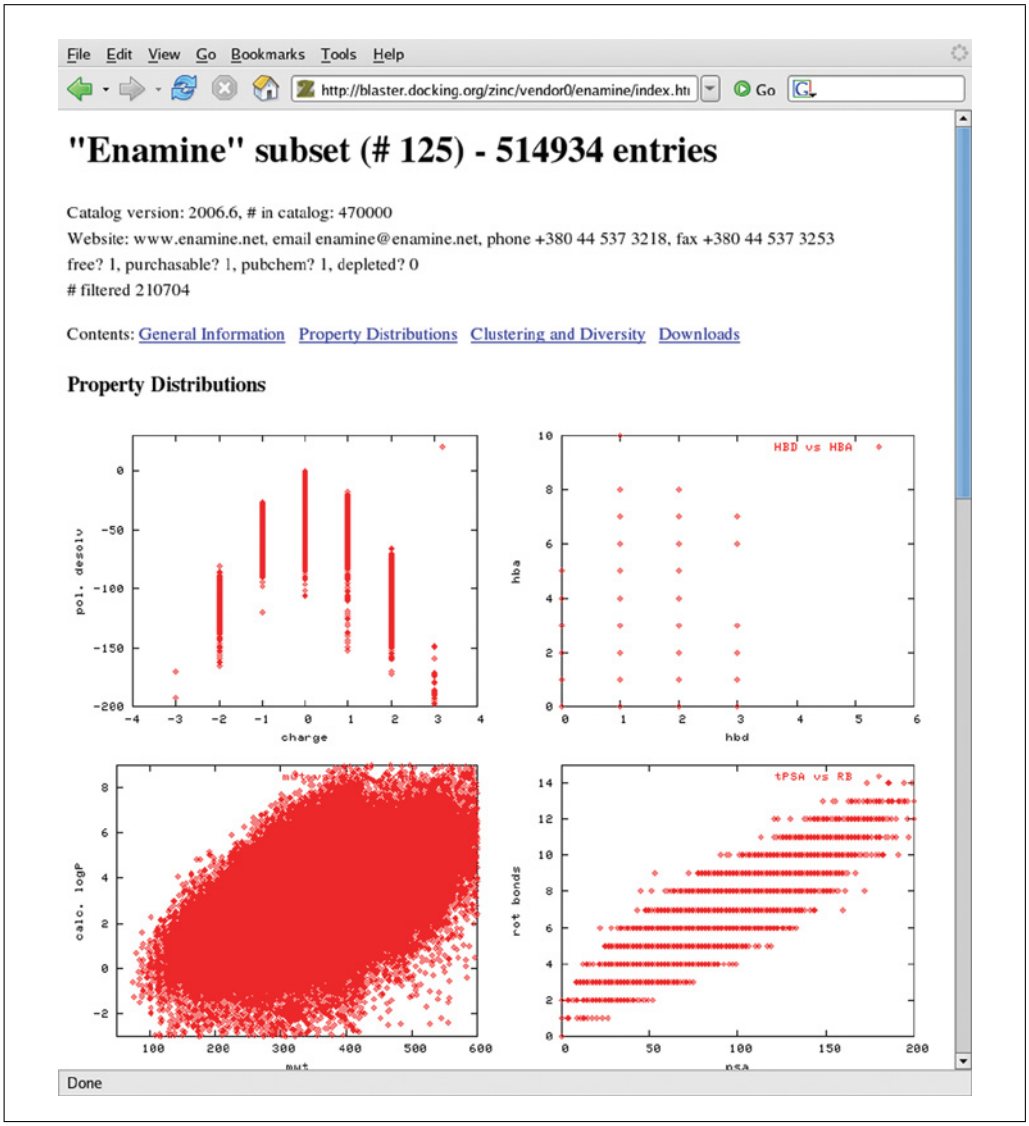

Figure 14.6.6 The ZINC database Enamine vendor download page.

7. Run the `usual.sdf.csh` script to download the database in compressed sdf format (see Basic Protocol 1, step 6).

8. If your docking program does not read gzip-compressed files, then you will need to uncompress the files before using them, using gunzip (see Basic Protocol 1, step 7).

At this point you have a 3-D dockable database of the Enamine "in stock" collection on your disk ready for screening. This protocol works equally well for all the vendors on the By Vendor page displayed in Figure 14.6.5. Some users may be interested in optional additional information about this subset, which is described below.

To download additional information about the Enamine subset of ZINC

9. Click on the last (bottom-most) link ("419986 compounds are ONLY available from a single vendor") on the download page (refer to Fig. 14.6.7).

This downloads a list of compounds only available from this vendor. Each row contains the ZINC ID and the original catalog number of the compound that is, according to our information, not available from any other vendor.

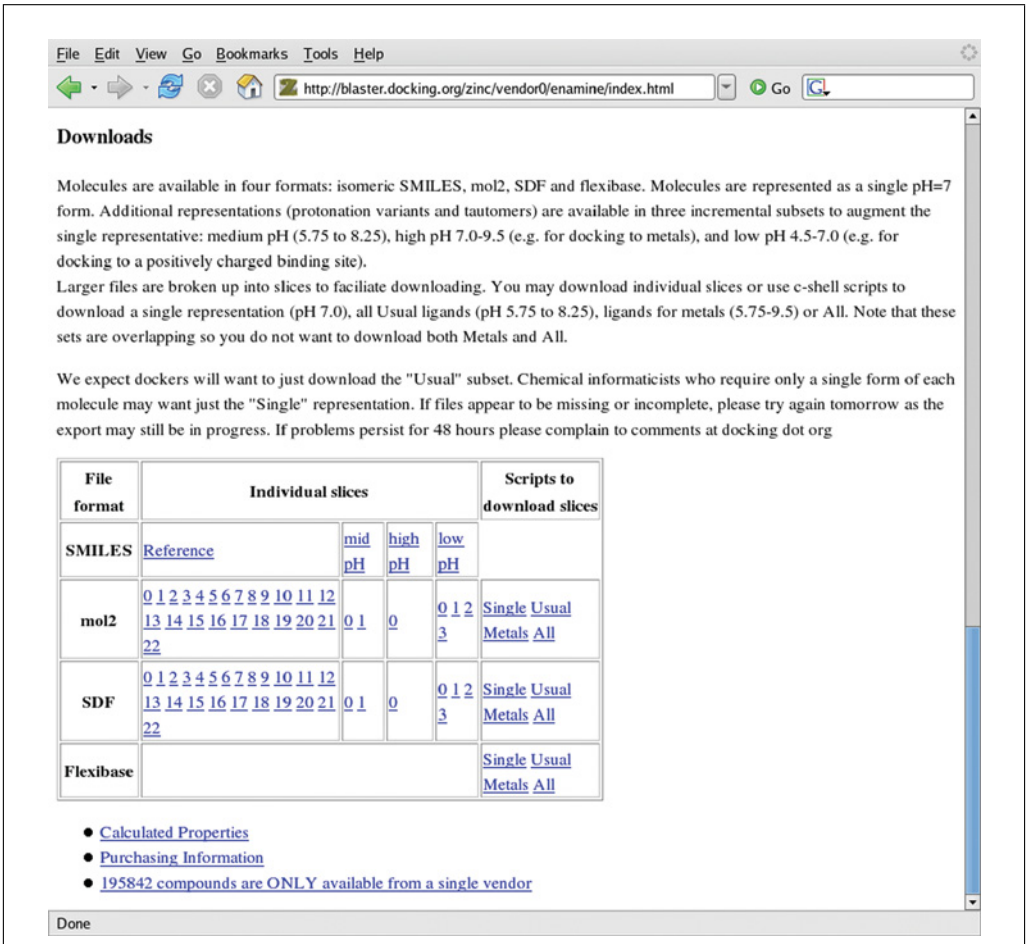
File Edit View Go Bookmarks Tools Help
http://blaster.docking.org/zinc/vendor0/enamine/index.html
Go
Downloads
Molecules are available in four formats: isomeric SMILES, mol2, SDF and flexibase. Molecules are represented as a single pH=7 form. Additional representations (protonation variants and tautomers) are available in three incremental subsets to augment the single representative: medium pH (5.75 to 8.25), high pH 7.0-9.5 (e.g. for docking to metals), and low pH 4.5-7.0 (e.g. for docking to a positively charged binding site).
Larger files are broken up into slices to faciliate downloading. You may download individual slices or use c-shell scripts to download a single representation (pH 7.0), all Usual ligands (pH 5.75 to 8.25), ligands for metals (5.75-9.5) or All. Note that these sets are overlapping so you do not want to download both Metals and All.
We expect dockers will want to just download the "Usual" subset. Chemical informaticists who require only a single form of each molecule may want just the "Single" representation. If files appear to be missing or incomplete, please try again tomorrow as the export may still be in progress. If problems persist for 48 hours please complain to comments at docking dot org
File format
Individual slices
Scripts to download slices
SMILES
Reference
mid pH
high pH
low pH
mol2
0 1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22
0 1
0
0 1 2 3
Single Usual Metals All
SDF
0 1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22
0 1
0
0 1 2 3
Single Usual Metals All
Flexibase
Single Usual Metals All
Calculated Properties
Purchasing Information
195842 compounds are ONLY available from a single vendor
Done

Figure 14.6.7 Bottom of the download page for vendor Enamine, featuring the download table.

10. Scroll to the Clustering and Diversity section of the Enamine download page (depicted at the top and bottom in Fig. 14.6.6 and Fig. 14.6.7, respectively), or click on the Clustering and Diversity link near the top of the page.

11. Click on the four entries in the table at the 60%, 70%, 80%, and 90% levels to download.

    This downloads cluster representatives as SMILES at four levels. For some applications, to save time, you may wish to download cluster representatives of the collection.

12. Click on Purchasing Information (second link from the bottom of the download page; see Fig. 14.6.7).

    This downloads a tab-delimited file of purchasing information to your computer for this subset. You can use this table to look up purchasing information without having to return to the ZINC Web site. The tab-delimited text file, which may be loaded into a spreadsheet, contains one row per catalog item as follows: catalog number, supplier name, contact information (Web site, e-mail, phone, and fax).

13. Click on Calculated Properties near the bottom of the download page (refer to Fig. 14.6.7).

    This downloads a tab-delimited text file containing calculated properties, one row per compound. Each row contains: ZINC ID, calculated logP, molecular weight, H-bond donors, H-bond acceptors, number of rotatable bonds, net charge, and polar surface area.

More information about this subset

14. Click the Back button in your browser to return to the table of available vendor subsets (Fig. 14.6.5).

15. In the Enamine row, click on the number of filtered-out compounds, which is the second line in the ZINC Information column.

    This downloads a list of compounds that were filtered out of the supplier catalog, one line per compound. For each compound, a reason is given as to why it was rejected. The rules for loading compounds into ZINC have evolved, and continue to change to reflect opinion in the field and our own biases. If a molecule in a supplier catalog is not in ZINC, look for it in this list. If you still want to have the molecule processed, you may process it yourself using Basic Protocol 5. We aim to provide a database that is useful to a broad audience, and thus you are welcome to suggest changes to our filtering rules by writing us comments at support@docking.org.

16. Click on the vendor's icon in the ZINC Database by Vendor page (Fig. 14.6.5).

    You may wish to browse the collection online before deciding to download it. To do this, click on the Enamine icon in the left-most cell.

17. From the Subset menu (on the Zinc homepage), select Synthesis on Request.

18. Proceed as if you were in the ZINC Database by Vendor section, described above in this protocol.

    Four vendors currently offer "synthesis on request" catalogs. These catalogs include compounds that can be made, often in <10 weeks, occasionally much faster. At this time there are more compounds available in ZINC via synthesis on request than there are in stock.

    When you have finished this protocol you will have a database of commercially available compounds from the vendor Enamine (or any other vendor you choose) that is ready to dock, screen, classify, or otherwise interpret. You may also have additional information if you performed any of steps 9 through 18. With such large files to download, errors in transmission may occur.

19. You may check that you successfully acquired the entire database subset by counting the number of unique ZINC ID numbers that are included in the files you downloaded, as follows:

```
unix> grep ZINC *.mol2 | sort -u | wc -l
```

20. If you have kept the files compressed, then use:

```
unix> zgrep ZINC *.mol2.gz | sort -u | wc -l
```

    The result of this command should be compared with the number of molecules in the subset, listed at the top of the download page. If the numbers differ by >1%, then compare the number of subset slices with the number in the `usual.sdf.csh` *script and with the number of slices listed on the download page. Incomplete, missing, or damaged files may be re-downloaded individually by clicking on the slice number in the download table at the bottom of the download Web page.*

BASIC PROTOCOL 3

SEARCHING ZINC

Use this protocol to explore the contents of ZINC online, and to find molecules that match some criteria. You should consider using this protocol if you already have a chemical starting point, for example, a list of actives, and you wish to identify similar commercially available molecules for screening, modeling, or acquisition. Directly finding molecules you like in ZINC can save time compared to downloading an entire subset such as the "lead-like" collection (Basic Protocol 1). You may search using molecular structure or substructure constraints expressed as SMILES or SMARTS, molecular property constraints, supplier catalog constraints, ZINC ID numbers, or any combination of these.

The result of a search is a list of molecules, which may be empty if none matches the criteria provided. This protocol illustrates the flexible search options available for finding molecules in ZINC. At the end of this protocol you should know how to specify molecular property, substructure, and other constraints to identify compounds of interest, browse them online, and download them, either individually or as a mini-subset. The ZINC search tool is limited: not all queries can be carried out in a single transaction. For example, to search for small rigid molecules, or medium-sized neutral molecules, it is advisable to perform this as two separate queries. For fastest performance, always try with "no time limit" unchecked at first, so that you get some feedback quickly. It is easy to go back a page in the browser and re-execute the query with no time limit after you are sure you are matching what you really want.

If you fail to find a particular molecule, or members of a particular class of molecule, even with "no time limit" checked, this may be due to one or more of the following reasons: (1) the pattern match may have failed, (2) the molecule may not be loaded, or (3) there may be a bug or a limitation in the ZINC system. Each of these three possibilities are discussed below.

The molecule you seek may not be in ZINC. One reason for this may be that the compound you seek may not be sold by the vendors that are used to build ZINC, even if the compound seems very popular. Even if it is in one of the source catalogs used for ZINC, it may have been filtered out or may have failed one of the many steps of processing. If a molecule you need is not in ZINC, you may attempt to process it using Basic Protocol 5. If this fails, or if you are stumped about the absence of a particular molecule, please contact us at *support@docking.org* to discuss.

Another reason the molecule may not be in ZINC is that there may be a problem with ZINC. For example, the search index may be out of date. There may be a transient problem with the ZINC server, the server may be undergoing maintenance, some key service may have crashed, a license may have expired, or there may be any number of other problems. If you suspect this is the reason, try to search again tomorrow. If you still do not find what you are looking for and you believe it is present in ZINC, please write us at *support@docking.org* to discuss.

Necessary Resources

Hardware

A modern computer with an Internet connection. Some files are very large, so 100 GB or more of free space may be required to store the uncompressed files in mol2 format.

Software

A Unix-like environment, such as Unix, Linux, Mac OS X, or Cygwin. See Support Protocol of *UNIT 9.6* for installation of Cygwin. Other operating systems may require minor changes.

If using Windows, wget is needed; this is available from SourceForge (*http://sourceforge.net/*) or the Web site *http://wget.docking.org/*. It may be easier to download ZINC files to a Unix-like machine and move the files to Windows.

A modern browser, such as Firefox 1.5 or later, Opera 9 or later, or Internet Explorer 7 or later (Internet Explorer 6 will work, barely, but is not advised), with the Java Runtime Environment (JRE). JRE is available from *http://java.sun.com/jre/* if not already installed.

Files

Some variants of this protocol may use a text file containing SMILES, SMARTS, or ZINC IDs, one item per line. SMARTS should be listed one per line with no

spaces or other characters. SMILES should have an integer Tanimoto similarity constraint between 0 and 100, after the SMILES, separated by whitespace.

To acquire molecules in ZINC similar to purine

1. Point the browser to *http://zinc.docking.org*.
2. Click on the Search and Browse link to go to the database search page (Fig. 14.6.8).

   Use this page for all ZINC database searches. On the left is a form for molecular property–based queries, which will be used later in the protocol. On the right, the Java Molecular Editor (JME) may be used to compose structure-based queries. The JME requires the Java Runtime Environment (JRE). Get the JRE from http://java.sun.com/jre/ if you do not have it.

3. For this example, we will search for molecules similar to purine. Draw purine in the JME (refer to Fig. 14.6.8 for the structure of purine). Click on the phenyl ring and then click in the drawing area. Click on cyclopentane and then click on one of the edges of the phenyl ring. Click on N and click on the four locations where N goes (refer to Fig. 14.6.8). Click on the double bond tool and click the bond in the five-membered ring to complete the aromatic system. If you make a mistake, you can click UDO to undo the last move or CLR to clear and start over.

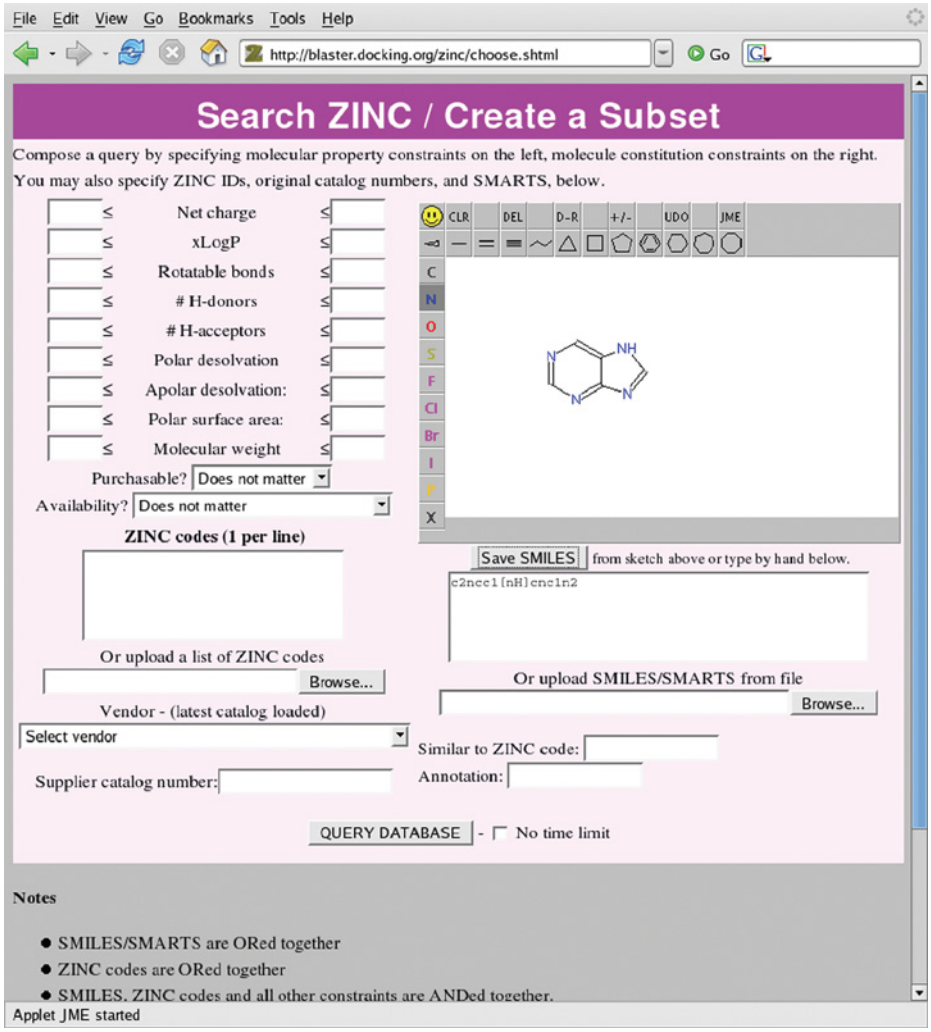
File Edit View Go Bookmarks Tools Help
http://blaster.docking.org/zinc/choose.shtml
Go
Search ZINC / Create a Subset
Compose a query by specifying molecular property constraints on the left, molecule constitution constraints on the right.
You may also specify ZINC IDs, original catalog numbers, and SMARTS, below.
Net charge
xLogP
Rotatable bonds
H-donors
H-acceptors
Polar desolvation
Apolar desolvation:
Polar surface area:
Molecular weight
Purchasable? Does not matter
Availability? Does not matter
ZINC codes (1 per line)
Or upload a list of ZINC codes
Browse...
Vendor - (latest catalog loaded)
Select vendor
Supplier catalog number:
CLR DEL D-R +/- UDO JME
C N O S F Cl Br I P X
Save SMILES from sketch above or type by hand below.
c2ncc1[nH]cnc1n2
Or upload SMILES/SMARTS from file
Browse...
Similar to ZINC code:
Annotation:
QUERY DATABASE - No time limit
Notes
SMILES/SMARTS are ORed together
ZINC codes are ORed together
SMILES, ZINC codes and all other constraints are ANDed together.
Applet JME started

Figure 14.6.8 Database Search and Browse page.

4. When you have finished, click on the Save SMILES link to write the SMILES code for purine in the window below the JME.

   It is the SMILES window and not the JME that is actually used for searching. The JME merely helps you compose the SMILES. You may use more than one SMILES, one per line. If you have difficulty creating the SMILES, you may type it manually as follows: `c2ncc1[nH]cnc1n2`. *(Please note the SMILES window is case sensitive.)*

5. Click your mouse after the SMILES for purine and type a space followed by `100`, indicating that you want to match this SMILES at or near the 100% Tanimoto similarity level (i.e., we seek identical or nearly identical molecules). To start the search, click the QUERY DATABASE button at the bottom of the page.

   The search may take 30 sec. When finished, your browser will display the molecules that have been found matching this pattern (Fig. 14.6.9), the first of which is purine.

   Processing speed depends on the load on the ZINC Web site servers, which varies minute to minute throughout the day. The default calculation returns a result after ∼30 sec, irrespective of load, which may result in an incomplete result. To force the server to perform an exhaustive search, click the No time limit checkbox to the right of the QUERY DATABASE button at the bottom of the search page (Fig. 14.6.8). In some cases, searches with no time limit may take a long time to complete. Note that the result contains molecules containing the purine ring substructure. You have thus just performed a Tanimoto similarity search using SMILES. SMILES is a powerful molecular specification language. You can read more at the Daylight Web site, http://www.daylight.com/dayhtml/doc/theory/index.html.

   Each molecule found that matches the search query appears as a separate row in the table, with a maximum of 500 rows at a time, three cells per row. Cell one contains the ordinal number in the list, the ZINC Id number, a "flag" icon used to indicate a problem to the curators, and a "discuss" button used to annotate or discuss this molecule on the wiki. The second cell contains purchasing information, molecular representation information, molecular properties, available annotations, any precalculated "Similar to" information and a Find Similar action button. The third cell has a 2-D depiction of the molecule, which opens a 3-D model when clicked.

   The result of this search is purchasing information for the compounds matching your query. You may also want to obtain additional information about this list of compounds, or about any one particular compound.

To view additional information about the compounds found in ZINC

6. Click on the MOL2 button at the top of the page (see Fig. 14.6.9).

   This downloads all the molecules matched (up to 500) in mol2 format.

7. Click on the Purchasing Info button at the top of the page.

   This downloads a table of purchasing information for all the molecules matched (up to 500) in tab-delimited text format, suitable for loading into a spreadsheet.

8. Click on the "Download table" button at the top of the page.

   This downloads a table of calculated molecular properties for all the molecules matched (up to 500) in tab-delimited text format, suitable for loading into a spreadsheet.

9. Click on the SMILES button at the top of the page.

   This downloads all the molecules matched (up to 500) as SMILES.

10. Click on the 2-D depiction of any molecule.

    This displays a 3-D structure of the molecule in a separate window, using a Java applet.

11. Click on the ZINC Id of the first hit, which is found in the left-most cell.

    This brings up a separate window containing only that molecule. It effectively focuses on a single molecule.

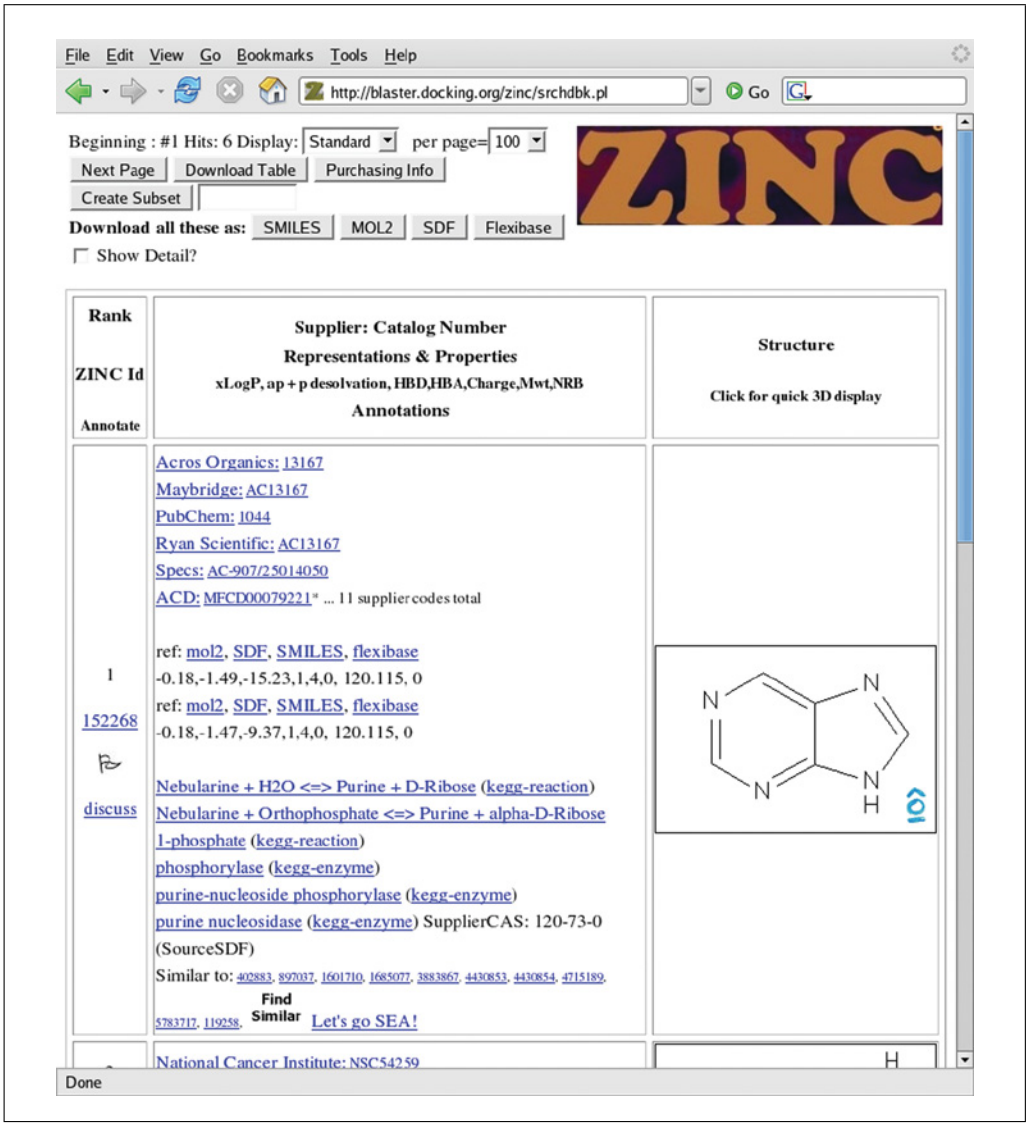

Figure 14.6.9 Results browser showing hits matching purine.

12. Click on the first "ref" link, called "mol2" for reference, in the second cell.

    This downloads the pH 7 representation of the molecule to your computer in mol2 format.

13. Click on the Find Similar button in the second cell.

    This searches ZINC in real time for similar molecules at the Tanimoto 80% similarity level. The search is exhaustive and may take anywhere from a few seconds to more than a minute, depending on the number of compounds matched, and the transient load on our servers.

14. Click on the Go SEA! button in the second cell (next to the Find Similar link).

    This scans the SEA database (Keiser et al., 2007) to see whether this molecule resembles any known drugs or metabolites.

15. Click on the catalog number next to the Supplier in the second cell.

    If the vendor has an e-commerce Web site, and if ZINC is aware of it and compatible with it, then you will be taken to the vendor's Web site where you may add the compound to your shopping basket on the vendor's site. If the foregoing conditions are not met, then you are offered an opportunity to write to the supplier enquiring about price and availability of the compound. Compounds may also be purchased via intermediary agents,

but this is not considered here. We also recommend using http://emolecules.com to check for information about price and availability, which is often faster and more current than ZINC.

To search for molecules containing a purine ring (via SMARTS)

16. Point the browser to *http://zinc.docking.org/*.
17. Click on the Search and Browse link to go to the database search page (Fig. 14.6.8).
18. Create purine (see step 3, above) in the JME and click Save SMILES.
19. Do not type 100 as you did in step 5 above. Without a number at the end of the line, this pattern will not be interpreted as SMARTS and will thus perform a substructure search.
20. Click on the QUERY DATABASE link at the bottom of the search page.

    The search may take up to 30 sec to run, depending on the load on our servers. The rest of the details are the same as above. The search result is now molecules that contain purine, not just those molecules that closely resemble purine.

21. If there are >500 molecules in the list (and there will be in this case), you may see the next page by clicking on Next Page at the upper-left-hand side of the browser page.

    To download >500 molecules at a time, you must create a subset (see Basic Protocol 4).

    You may experiment with different values of Tanimoto similarity, the molecule used to search. You may enter multiple SMILES in the same line, separated by the conjunction ".", which means that each of them must match (logical AND).

To search ZINC using physicochemical property constraints

22. Point your browser to *http://zinc.docking.org*.
23. Click on the Search and Browse link to the database search page (Fig. 14.6.8).
24. To find small, neutral, rigid molecules, enter numbers as follows. In Rotatable bonds and Net charge, enter `0` as both high and low constraints. For Molecular weight, enter `50` as a lower bound and `100` as the maximum constraint.
25. Click on the QUERY DATABASE link at the bottom of the search page.

    In a few seconds the results appear in your browser. Proceed to browse these results as described above in steps 6 to 15.

To retrieve ZINC entries by ZINC ID number

26. Point the browser to *http://zinc.docking.org*.
27. Click on the Search and Browse link to the database search page (Fig. 14.6.8).
28. In the ZINC codes field (left-hand side, center) type the number `1234567`. (You may enter more than one ZINC ID, one per line).
29. Click on the QUERY DATABASE link at the bottom of the search page.

    In a few seconds the results appear in your browser. Proceed to browse these results as described above in steps 6 to 15.

CREATE AND DOWNLOAD A CUSTOM SUBSET

BASIC PROTOCOL 4

As we have seen, you may download large preprepared subsets by property (Basic Protocol 1) or vendor (Basic Protocol 2). You may also download mini-subsets of up to 500 molecules directly from the results of a ZINC database search (Basic Protocol 3). If you would like to download >500 but <10,000 molecules based on your own search

criteria, then you should use this protocol. If you require a subset with >10,000 molecules, please write to us to request a custom subset be made for you. Basic Protocol 4 is only supported in ZINC version 8.

Necessary Resources

Hardware

A Unix-like environment, such as Unix, Linux, Mac OS X, or Cygwin. See Support Protocol of *UNIT 9.6* for installation of Cygwin. Other operating systems may require minor changes.

Software

If using Windows, wget is needed; this is available from SourceForge (*http://sourceforge.net/*) or the Web site *http://wget.docking.org/*. It may be easier to download ZINC files to a Unix-like machine and move the files to Windows.

A modern browser, such as Firefox 1.5 or later, Opera 9 or later, or Internet Explorer 7 or later (Internet Explorer 6 will work, barely, but is not advised), with the Java Runtime Environment (JRE). JRE is available from *http://java.sun.com/jre/* if not already installed.

To create a custom subset of purine-ring containing compounds that have logP <4 and have molecular weight <400

1. Point the browser to *http://zinc8.docking.org/*.
2. Select Search and Browse from the Home pull-down menu.
3. Specify the search as in Basic Protocol 3, sketching purine, clicking Save SMILES, and adding constraints for logP <4 and molecular weight <400.
4. Click on the QUERY DATABASE link at the bottom of the search page.
5. At the top of the listing of results click on the Create Subset link.

   After a brief pause of no more than a minute, a message reading "Creating subset X. Browse subset X. Browse user-created subsets." appears, where X is the number of the subset being created. Subset preparation typically takes ~1 min per 100 molecules, with a 1 min minimum. You may browse all currently available subsets by clicking on Browse user-created subsets or by choosing User Subsets from the Subsets pull-down menu at any time.

   The subset download page contains a directory listing of all files, and a helpful guide to the most useful files at the bottom of the page. Click on the files that match the pattern `e_p0.*.mol2.gz` *to get the reference structures at pH 7 in mol2 format and on* `e_p1.mol2.gz` *to get additional protonated and tautomerized forms near physiological pH.*

   Subset preparation is a live service, and thus may fail for various reasons. Although most subsets are ready in minutes, please wait 24 hr before reporting a problem so that we can attempt to fix it first. If you find us slow, there is a link where you may bring a failure to our attention.

BASIC PROTOCOL 5

UPLOAD AND PROCESS YOUR OWN MOLECULES

Sometimes you want to dock molecules that are not in ZINC. These could be molecules you have made, or are considering making. This protocol describes how to process arbitrary molecules. A maximum of 1000 molecules may be processed in each transaction. Some restrictions apply (see Table 14.6.1). Compounds are filtered, and will be rejected, with reasons, if they do not pass. This facility is not for vendors to upload their catalogs. Vendors, please contact *support@docking.org* to send us your catalogs in SDF format,

Table 14.6.1 Limitation to Uploading and Processing Molecules

Limitation	Comments
File formats for Basic Protocol 5	Basic Protocol 5 depends on receiving correctly formatted files of molecules. Prospective users of this service should pay attention to the standards for SMILES, SDF, and mol2 formats.
Subset limitations	You may only create subsets of up to 10,000 molecules.
Filtering restrictions	ZINC filters molecules to prevent molecules that we think are unlikely candidates for structure-based ligand discovery from being loaded. The filtering rules continue to evolve with our research. The current rules in effect are available on our Web site, *http://filtering.docking.org*.
Failure during upload	File upload and processing is perhaps the most error-prone service offered on the ZINC Web site. This is because success depends on many factors: the file format of the data being uploaded, the chemical constitution of the molecules themselves, the availability of our servers, and the correctness of our processing scripts. Normally, an error message is produced by failures, and we review these messages regularly. If you can't wait for us, or if you think we have not noticed the failure, please bring the failure to our attention.

which we will process and load ourselves. Basic Protocol 5 is only supported in ZINC version 8.

Necessary Resources

Hardware

A Unix-like environment, such as Unix, Linux, Mac OS X, or Cygwin. See Support Protocol of *UNIT 9.6* for installation of Cygwin. Other operating systems may require minor changes.

Software

If using Windows, wget is needed; this is available from SourceForge (*http://sourceforge.net/*) or the Web site *http://wget.docking.org/*. It may be easier to download ZINC files to a Unix-like machine and move the files to Windows.

A modern browser, such as Firefox 1.5 or later, Opera 9 or later, or Internet Explorer 7 or later (Internet Explorer 6 will work, barely, but is not advised), with the Java Runtime Environment (JRE). JRE is available from *http://java.sun.com/jre/* if not already installed.

Files

You will need the molecules to be formatted in either SMILES, mol2, or SDF format. We recommend SMILES format. We will convert your mol2 and SDF to SMILES before generating 3-D structures.

View and download additional information about the "lead-like" subset

1. Point the browser to *http://zinc8.docking.org/*.
2. Select User Upload from the Home pull-down menu.
3. Click on the Browse link to select your file in either SMILES, mol2, or SDF format. (Fig. 14.6.10).

   All other fields are optional, and will only be used to annotated the uploaded ligands.

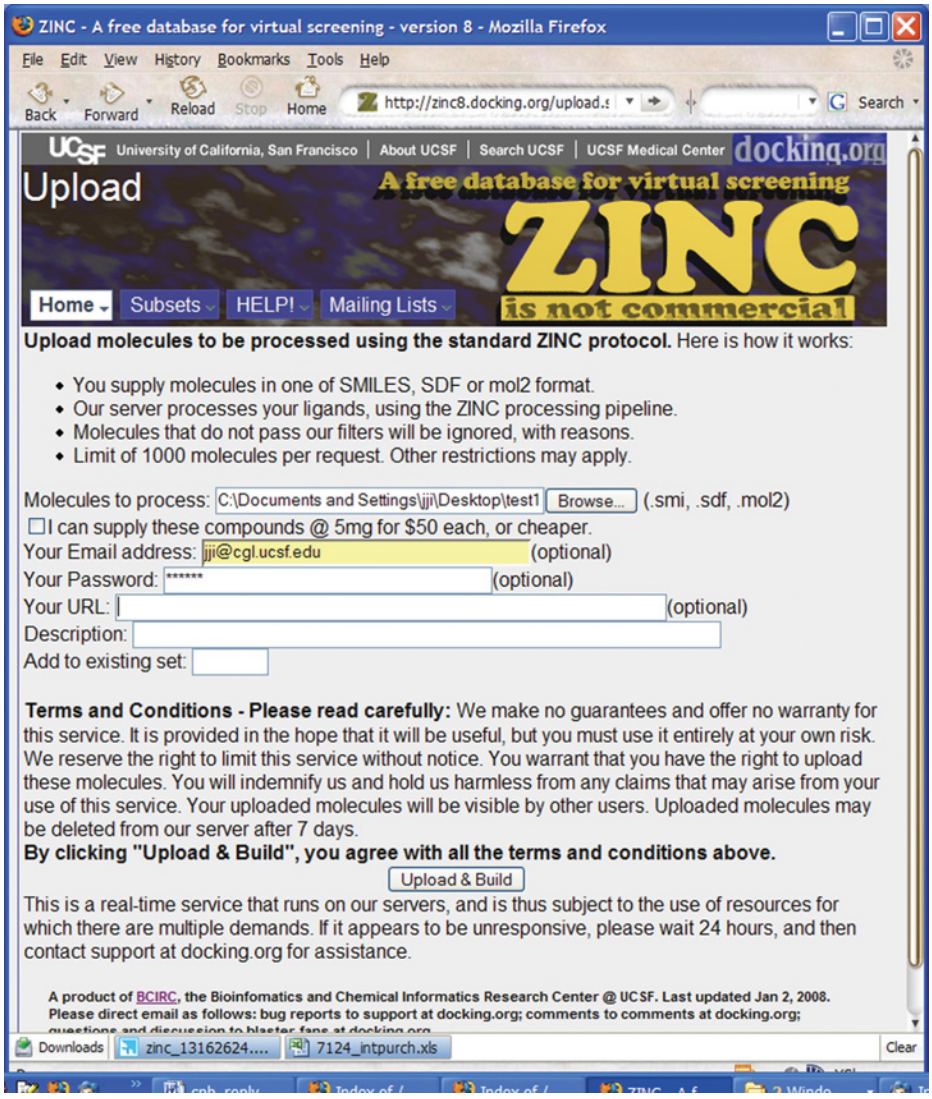
ZINC - A free database for virtual screening - version 8 - Mozilla Firefox
File Edit View History Bookmarks Tools Help
Back Forward Reload Stop Home
http://zinc8.docking.org/upload.s
Search
UCSF University of California, San Francisco | About UCSF | Search UCSF | UCSF Medical Center
docking.org
Upload
A free database for virtual screening
ZINC
is not commercial
Home Subsets HELP! Mailing Lists
Upload molecules to be processed using the standard ZINC protocol. Here is how it works:
You supply molecules in one of SMILES, SDF or mol2 format.
Our server processes your ligands, using the ZINC processing pipeline.
Molecules that do not pass our filters will be ignored, with reasons.
Limit of 1000 molecules per request. Other restrictions may apply.
Molecules to process: C:\Documents and Settings\jji\Desktop\test1 Browse... (.smi, .sdf, .mol2)
I can supply these compounds @ 5mg for $50 each, or cheaper.
Your Email address: jji@cgl.ucsf.edu (optional)
Your Password: ****** (optional)
Your URL: (optional)
Description:
Add to existing set:
Terms and Conditions - Please read carefully: We make no guarantees and offer no warranty for this service. It is provided in the hope that it will be useful, but you must use it entirely at your own risk. We reserve the right to limit this service without notice. You warrant that you have the right to upload these molecules. You will indemnify us and hold us harmless from any claims that may arise from your use of this service. Your uploaded molecules will be visible by other users. Uploaded molecules may be deleted from our server after 7 days.
By clicking "Upload & Build", you agree with all the terms and conditions above.
Upload & Build
This is a real-time service that runs on our servers, and is thus subject to the use of resources for which there are multiple demands. If it appears to be unresponsive, please wait 24 hours, and then contact support at docking.org for assistance.
A product of BCIRC, the Bioinfomatics and Chemical Informatics Research Center @ UCSF. Last updated Jan 2, 2008.
Please direct email as follows: bug reports to support at docking.org; comments to comments at docking.org;
Downloads
zinc_13162624....
7124_intpurch.xls
Clear

Figure 14.6.10 Database Upload page.

4. Click Upload & Build to start the process.

   You will receive a message indicating that the molecules have been uploaded and that they are being processed. (Fig. 14.6.11). Processing can take up to a minute per molecule, with a minimum of 1 min. You may browse to the ligands. As in Basic Protocol 4, there is a helpful guide to the important files at the bottom of the download page. You may download the mol2 representations at pH 7 using `e_p0.*.mol2.gz` *and the additional forms near physiological pH using* `e_p1.*.mol2.gz`.

   The directory contains a report about molecules that were filtered out (`filterlog.txt`*), with justifications. There is a log file of the processing (*`stdout, stderr`*), which may contain messages about problems during processing. There are three files mapping your identification numbers to ZINC identification numbers depending on whether they were already in ZINC (*`alreadyinzinc.smi, inzinc.smi`*) or were processed for the first time (*`dict`*).*

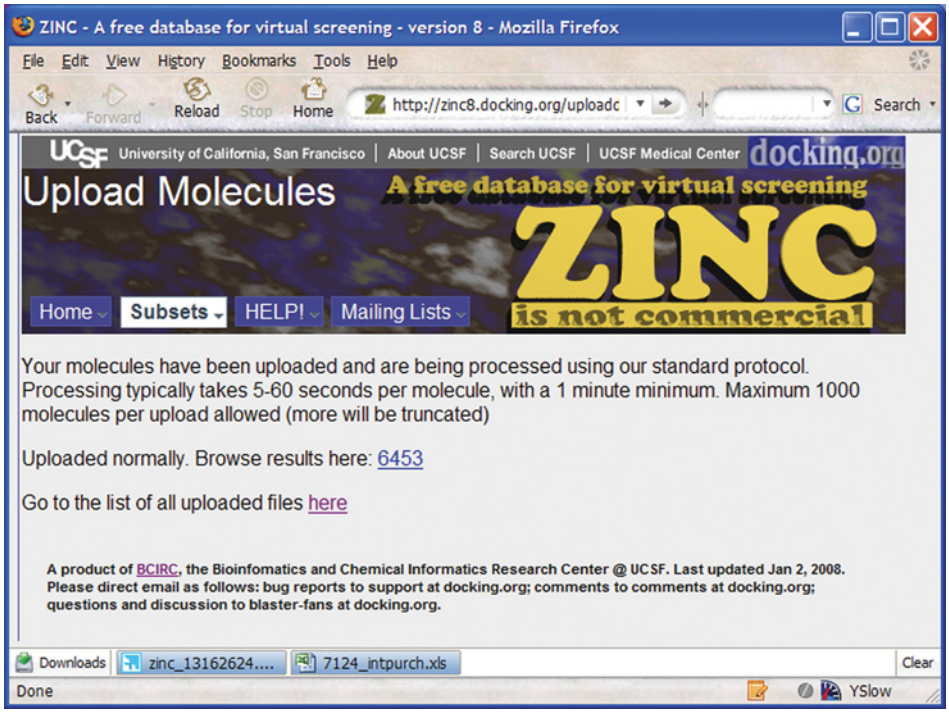
ZINC - A free database for virtual screening - version 8 - Mozilla Firefox
File Edit View History Bookmarks Tools Help
Back Forward Reload Stop Home
http://zinc8.docking.org/uploadc
Search
UCSF University of California, San Francisco | About UCSF | Search UCSF | UCSF Medical Center
docking.org
Upload Molecules
A free database for virtual screening
ZINC
is not commercial
Home Subsets HELP! Mailing Lists
Your molecules have been uploaded and are being processed using our standard protocol.
Processing typically takes 5-60 seconds per molecule, with a 1 minute minimum. Maximum 1000 molecules per upload allowed (more will be truncated)
Uploaded normally. Browse results here: 6453
Go to the list of all uploaded files here
A product of BCIRC, the Bioinfomatics and Chemical Informatics Research Center @ UCSF. Last updated Jan 2, 2008.
Please direct email as follows: bug reports to support at docking.org; comments to comments at docking.org;
questions and discussion to blaster-fans at docking.org.
Downloads
zinc_13162624....
7124_intpurch.xls
Clear
Done
YSlow

Figure 14.6.11 Page showing Upload & Build status.

COMMENTARY

Background Information

ZINC was created to lower one of the barriers to entry to virtual screening: the preparation of a 3-D database suitable for docking. ZINC serves this purpose by processing catalogs from many of the most important compound vendors and making ready-to-dock databases available in easy-to-download formats. ZINC is organized for download by physicochemical properties (Basic Protocol 1) and vendor (Basic Protocol 2). Search facilities (Basic Protocol 3) enable browsing and the download of small (<500 molecules) subsets. Mid-size subsets containing up to 10,000 molecules may be created and downloaded just like preprepared subsets (Basic Protocol 4). Larger subsets may be created only on request to *support@docking.org* as this can entail significant CPU usage and possibly some curation. Finally, there will always be molecules that are not in ZINC, so small sets of molecules (<1000) may be uploaded for processing using our standard pipeline (Basic Protocol 5).

Vendor catalogs evolve over time. Some vendors make as many as 20,000 new compounds in a single month! Similarly, every quarter tens of thousands of molecules become depleted and can no longer be purchased. ZINC aims to keep up with this staggering rate of change, at least annually, and, one day perhaps, faster than that. Moreover, we try to add several new vendors every year. Sadly, some vendors occasionally cease operations, requiring updating of our records. At any one time, it is typical that perhaps 20% of selected compounds from a virtual screen may no longer be purchasable. We therefore recommend that you select ~20% more compounds than you aim to buy to avoid disappointment. Supply rates do vary by vendor, but much of this data is anecdotal, and also changes over time.

The ZINC pipeline makes use of third-party software from collaborative software vendors. The ZINC protocol attempts to incorporate the latest tested algorithms from these vendors, and as a result, the treatment of molecules changes gradually over time. Keeping ZINC curated and correct is a never-ending process, and we acknowledge that there are numerous "broken" or otherwise problematic molecules in ZINC. If you find something, please tell us. We attempt to fix all problems that are reported to us.

Biologically relevant forms are important for physics-based scoring of docked poses, and are a central feature and organizing principle of ZINC. Besides 3-D docking applications, ZINC has been used for chemical informatics

(2-D) applications. In this case, we recommend the use of the "pH 7" or "single" representation of the database.

Critical Parameters and Troubleshooting

Corrupt or incomplete files

If a file you acquired from the ZINC Web site seems incomplete or damaged in some way, or cannot be uncompressed, or does not look right for some reason, the first thing to try is to redownload the file manually. If the file is still corrupt, please contact us at *support@docking.org* to correct it. We offer tens of millions of separate files for download, and we would be surprised if there were no problems at all. Please bring problems to our attention and we will try to fix them as soon as logistics allow.

Search, Upload, Subset creation or Web page browsing hung

If a search seems to run forever, try without the No time limit option checked. If it still runs forever, there may be a problem with our servers. Please be patient, and try again later. If you can't wait, or if it seems persistently hung, please email us at *support@docking.org* describing the problem you are seeing.

Problems with the interface and how to report them

The ZINC Web site continues to develop and evolve. Numerous errors and other problems have been reported in the ZINC Web site since it first appeared 2 years ago. Moreover, transient errors due to system load, full disks, or failed components occur from time to time. If you notice a problem with the ZINC Web site, please report it to us at *support@docking.org* and we will do our best to fix it as soon as logistics allow.

Acknowledgments

This work was supported by NIGMS, GM71896 (to Brian K. Shoichet and JJI). We thank participating compound suppliers, which are named on the ZINC Database by Vendor Web page. We are grateful to our commercial software suppliers for access to their software and technical support: OpenEye Scientific Software (*http://www.eyesopen.com*), Xemistry GmbH (*http://xemistry.com*), Molinspiration (*http://www.molinspiration.com*), Molecular Networks (Germany), and Schrödinger Inc. (*http://www.schrodinger.com*).

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Keiser, M.J., Rother, B.L., Armbruster, B.N., Ernsberger, P., Irwin, J.J., and Shoichet, B.K. 2007. Relating protein pharmacology by ligand chemistry. *Nat. Biotechnol.* 25:197-206.

Key References

Huang, N., Shoichet, B.K., and Irwin, J.J. 2006. Benchmarking sets for molecular docking. *J. Med. Chem.* 49:6789-6801.

Irwin, J.J. 2006. How good is your screening library? *Curr. Opin. Chem. Biol.* 10:352-356.

Irwin, J.J. and Shoichet, B.K. 2005. ZINC: A database of commercially available compounds for virtual screening. *J. Chem. Inf. Model* 45:177-182.

This paper describes the original public release of the ZINC database (version 4, released January 2005).

Internet Resources

http://zinc.docking.org/

The Zinc Web site. All the protocols described in this unit use the ZINC Web site exclusively.

http://daylight.com

The Daylight Theory Manual describes SMARTS and SMILES.

http://dock.compbio.ucsf.edu

DOCK is an example of a docking program that can use molecules from ZINC.

http://emolecules.com

Commercially available chemical space, without the 3-D representation.

http://pubchem.ncbi.nlm.nih.gov/

Source of pubchem molecules.

http://eyesopen.com

Source of the OEChem chemical informatics toolkit, the Omega conformational sampling program, the Ogham depiction tools, the QuacPAC charge and electrostatics tools, and other tools used by ZINC. Also home of VIDA, a remarkable visualization tool, that works well with ZINC.

http://xemistry.com

Source of Cactvs, used to prepare ZINC and to support numerous key functions on the ZINC Web site.

http://molinspiration.com

Source of mitools, used to calculate molecular properties for ZINC. Mitools are noteworthy for catching errors in SMILES that other packages miss.

http://www.schrodinger.com

Source of ligprep, used to protonate and tautomerize molecules in ZINC.

http://comp.chem.umn.edu/amsol/

Source of AMSOL, the semiempirical quantum mechanics program by Cramer and Truhlar with a salvation-adjusted Hamiltonian, used to calculate partial atomic charges and atomic desolvation penalties in ZINC.

PharmGKB: An Integrated Resource of Pharmacogenomic Data and Knowledge

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ABSTRACT

The PharmGKB is a publicly available online resource that aims to facilitate understanding how genetic variation contributes to variation in drug response. It is not only a repository of pharmacogenomics primary data, but it also provides fully curated knowledge including drug pathways, annotated pharmacogene summaries, and relationships among genes, drugs, and diseases. This unit describes how to navigate the PharmGKB Web site to retrieve detailed information on genes and important variants, as well as their relationship to drugs and diseases. It also includes protocols on our drug-centered pathway, annotated pharmacogene summaries, and our Web services for downloading the underlying data. Workflow on how to use PharmGKB to facilitate design of the pharmacogenomic study is also described in this unit. *Curr. Protoc. Bioinform.* 23:14.7.1-14.7.17. © 2008 by John Wiley & Sons, Inc.

Keywords: database • pharmacogenomics • pharmacogenetics • drug response • genetic variation • pathway analysis • SNP • polymorphisms • study design

INTRODUCTION

Pharmacogenomics is the study of how genetic variation contributes to variation in drug response. Driven by technology advancements in the post-genomic era, pharmacogenomics research has the potential to optimize drug efficacy and minimize toxicity. It bridges the gap between the scientific discoveries and clinical application, and offers the exciting promise of personalized drug therapy. The Pharmacogenetics and Pharmacogenomics Knowledge Base (PharmGKB) is a publicly available internet resource for pharmacogenomic data and knowledge (Klein and Altman, 2004). PharmGKB strives to capture rapid advancements in the pharmacogenomics area. It is the central data repository for pharmacogenetic and pharmacogenomic data, in addition to providing integrated knowledge including drug pathways, gene summaries, and relationships among genes, drugs, and diseases.

PharmGKB serves diverse user groups from the scientific community. It provides comprehensive and integrated drug, gene, and disease information to pharmacologists, clinical investigators, and biologists, as well as to informaticians. The PharmGKB homepage has been designed in a way that highlights the primary interests of most users, and registered users have complete access to individualized genotype and phenotype data for discovery research and further analysis. PharmGKB is also an excellent educational portal for any person who is new to pharmacogenomics. A graphic schema depicting the central elements involved in drug response and associated genetic basis is displayed on the PharmGKB homepage. Also provided on the homepage are lecture materials, tutorials, and useful links intended to help people familiarize themselves with the fundamental concepts of pharmacogenomics research and personalized medicine.

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The protocols in this unit describe how to use PharmGKB to browse pharmacogenomic data and knowledge. The Basic Protocol describes how to navigate the PharmGKB homepage and browse through the knowledge base starting from the search by gene option. Support Protocol 1 explains in detail how to use our variant browser and variant table; Support Protocol 2 describes how to explore our unique drug-centered pathways; Support Protocol 3 demonstrates the types of knowledge contained in our Very Important Pharmacogene (VIP) gene summaries; and Support Protocol 4 describes our Web services project, which allows our users to bulk download data from PharmGKB.

BASIC PROTOCOL

NAVIGATING THE HOMEPAGE OF PharmGKB USING SEARCH BY GENE

This protocol introduces the basic techniques used for searching and browsing the content on the PharmGKB Web site.

Necessary Resources

Hardware

Computer with an internet connection

Software

Any up-to-date Web browser will work.

Files

No input files required

The PharmGKB homepage: Getting started

1. Open the PharmGKB homepage at *http://www.pharmgkb.org* in a Web browser.

   The PharmGKB homepage is the common entry point for all users. It has been designed to highlight the types of information that are most sought after by a diverse group of users. A graphic schema for understanding the basis of pharmacogenomics has also been included (Fig. 14.7.1).

   The menu tabs at the top of the page provide access to the top-level section of the PharmGKB site; see Table 14.7.1 for a description of the various tabs. Prominently displayed in the center of the homepage are clickable icons that allow our users to go directly to a specific type of pharmacogenomic-related data, such as pathways, genes, variants of interest, drugs, diseases, and download information. Right below the icon is the search box where a user can enter text for a Google-type query. The search box is also prominently displayed at the top right-hand corner of the homepage. At the top right of every PharmGKB page, a feedback link is available. A scientific curator responds to all feedback within 48 hr.

   The PharmGKB homepage also provides basic tutorial information about pharmacogenetics and pharmacogenomics. Below the search box is a graphic schema that illustrates the basic flow of pharmacogenetic information. After a drug is administered, it is absorbed, distributed, metabolized, and excreted (pharmacokinetics; PK); the drug then reaches its target and elicits a drug response (pharmacodynamic effects; PD). Both the PK and PD of the drug can be influenced by an individual's genetic makeup (GN) and in turn, lead to distinct clinical outcomes (CO). The five categories of evidence (COE) mentioned above appear in this diagram, and their relationship to each other is indicated in the picture. Definitions of these terms are provided in the Useful Links section at the bottom-right of the homepage. Another valuable learning resource on the PharmGKB homepage is the list of Curators' Favorite Papers. This is a biweekly feature that covers recent "hot" papers in pharmacogenomics. Each paper is annotated with the pertinent categories of evidence (COE) and tagged with relevant genes, drugs, and diseases.

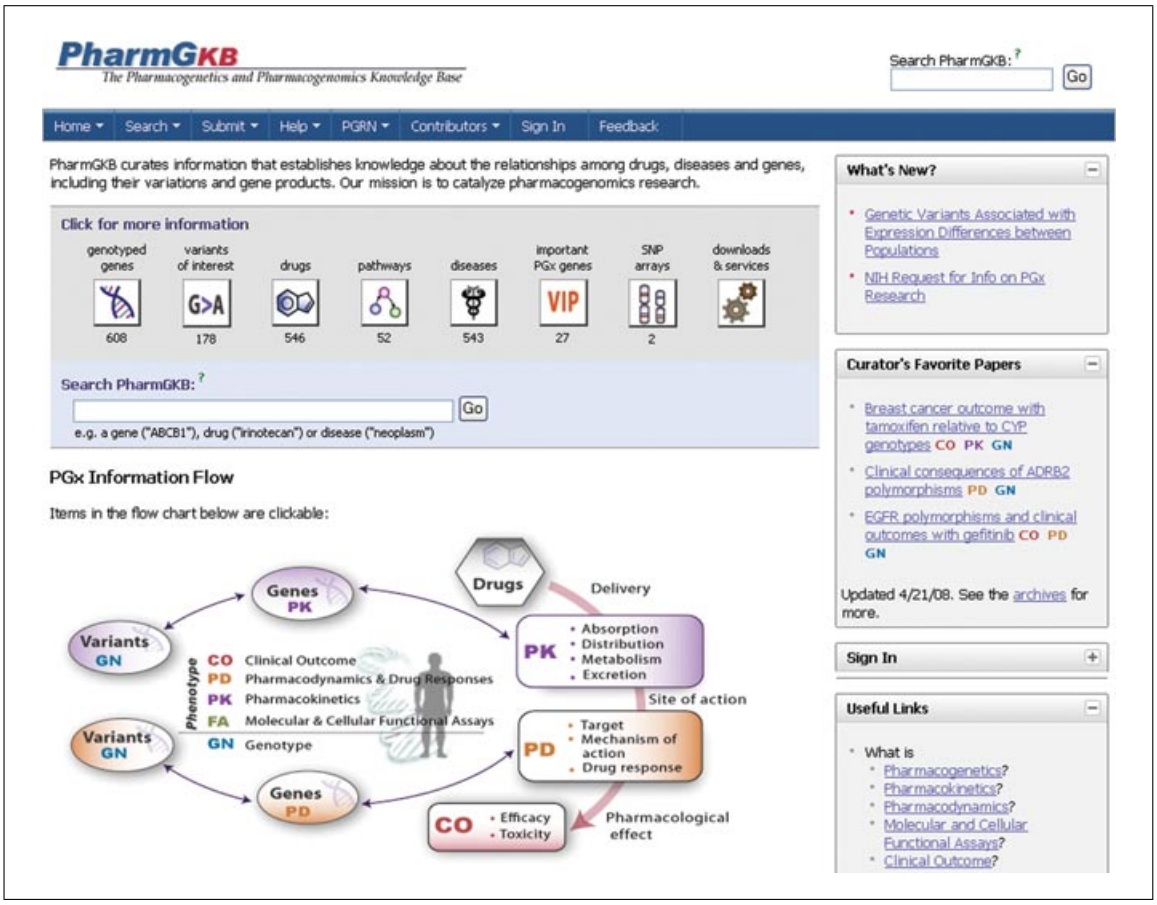


Figure 14.7.1 The PharmGKB homepage (*http://www.pharmgkb.org*).

Table 14.7.1 Description of Menu Tabs

Tab	Description
Home	The front page where we highlight our knowledge and data content, mission, contact information, and registration
Search	The main search page where we can either search by free text, user-canned queries, or browse information by domain
Submit	The section that describes how a user can submit genotype, phenotype, pathway, or literature data
Help	An extensive list of background information, downloads, educational, as well as technical references
PGRN	Lists all members involved in the NIH Pharmacogenetics Research Network, their research interests, and submissions to PharmGKB
Contributors	The section where people are listed who have contributed data to PharmGKB
My PharmGKB	The section for our registered users to view their profile, submission, and Web site statistics

Searching by Gene and its associated variant, pathways, drugs, and disease information

A gene can be searched by either typing the gene name or symbol in the search box, or, clicking on the gene icon from the homepage and then browsing through the alphabetically sorted gene list. For example, to search for VKORC1, a key protein in vitamin K metabolism and target of the anticoagulant drug warfarin, type `VKORC1` in the search

PharmGKB
The Pharmacogenetics and Pharmacogenomics Knowledge Base
Search PharmGKB:
Go
Home
Search
Submit
Help
PGRN
Contributors
Sign In
Feedback
VKORC1
vitamin K epoxide reductase complex, subunit 1
Overview
VIP
Variants
Datasets
Pathways
Curated Publications
Downloads/Cross-references
Overview
PharmGKB Accession Id: PA133787052
Alternate Names: VKCFD2; Vitamin K-dependent clotting factors, combined deficiency of, 2; phylloquinone epoxide reductase; vitamin K dependent clotting factors deficiency 2; vitamin K1 epoxide reductase (warfarin-sensitive)
Alternate Symbols: EDTP308; FLJ00289; IMAGE3455200; MGC2694; MST134; MST576; UNQ308; VKCFD2; VKOR
Details
Cytogenetic Location: chr16 : p11.2
GP mRNA Boundary†: chr16 : 31003777 - 31012676
GP Gene Boundary†: chr16 : 31003777 - 31018002
Strand: minus
OMIM Phenotype: Vitamin K-dependent clotting factors, combined deficiency of, 2, Warfarin resistance
Product Name: Vitamin K-dependent clotting factors, combined deficiency of, 2, phylloquinone epoxide reductase, vitamin K dependent clotting factors deficiency 2, vitamin K epoxide reductase complex, subunit 1, vitamin K epoxide reductase complex, subunit 1 isoform 1, vitamin K epoxide reductase complex, subunit 1 isoform 2, vitamin K1 epoxide reductase (warfarin-sensitive)
† The mRNA boundaries are calculated using the gene's default feature set from NCBI, mapped onto Golden Path. The gene boundaries are set by PharmGKB by expanding the mRNA boundaries to allow for upstream and downstream regulatory regions.

Figure 14.7.2 Example of PharmGKB Gene page (VKORC1, vitamin K epoxide reductase complex, subunit 1).

box, then click "go." If no result is returned, try a synonym or partial name. Both alternative names and other symbols that might have been used in the literature for the gene of interest are included for all genes in PharmGKB. We adhere to the nomenclature at HUGO Gene Nomenclature Committee (HGNC) for official gene names (Eyre et al., 2006), and make every effort to keep them current.

2. Open the VKORC1 gene page (Fig. 14.7.2).

   The main gene page is also organized by a tab system similar to the homepage. The overview tab lists the alternative gene symbols and gene names as well as details, such as gene and mRNA boundaries, and their OMIM phenotype, if available. Additional tabs for the gene include Datasets, Pathways, Curated, and noncurated publications. The last tab is the downloads/cross-references, which illustrates the links to download genotype or phenotype data associated with the gene. It also lists unique identifiers used by PharmGKB and other external genomic databases for the specific gene.

3. Click on the VIP tab to view the VKORC1 VIP gene summary containing detailed information on variant and haplotype mapping and their importance in drug responses. (See Support Protocol 3 for details).

4. Click on the Variants tab to display all the variants for VKORC1 available in PharmGKB in the browser, as well as in the variant table with variant details and functional annotation for variants of interests (see Support Protocol 1 for details).

5. To view curated phenotype data associated with VKORC1, click on the Datasets tab, then select the link titled WUSTL warfarin dosing data, group A (Fig. 14.7.3).

   Phenotype data at PharmGKB are organized by a tab system, similar to that on the homepage. The Overview tab lists the investigator, related genes, drugs, and disease, as well as a summary for the study. The second tab, Publications, lists all publications related to that phenotype. The third tab lists all column headers and descriptions for the

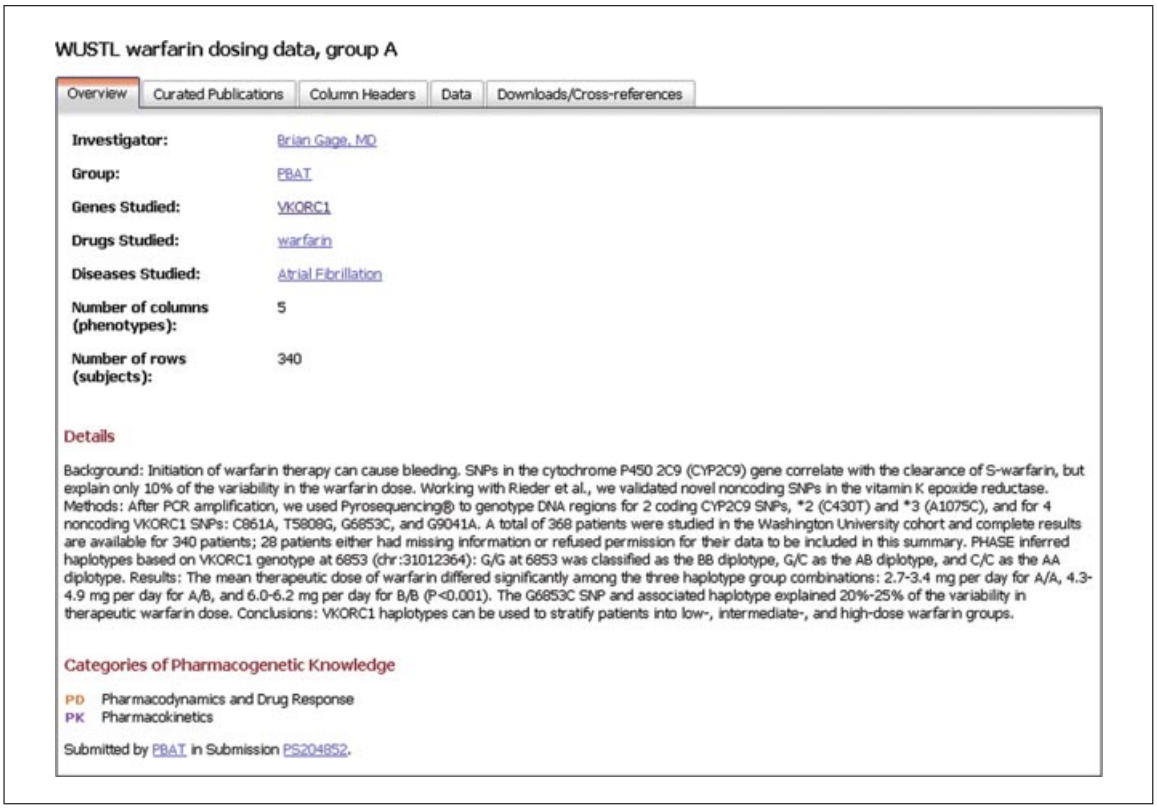

WUSTL warfarin dosing data, group A
Overview
Curated Publications
Column Headers
Data
Downloads/Cross-references
Investigator:
Brian Gage, MD
Group:
PBAT
Genes Studied:
VKORC1
Drugs Studied:
warfarin
Diseases Studied:
Atrial Fibrillation
Number of columns (phenotypes):
5
Number of rows (subjects):
340
Details
Background: Initiation of warfarin therapy can cause bleeding. SNPs in the cytochrome P450 2C9 (CYP2C9) gene correlate with the clearance of S-warfarin, but explain only 10% of the variability in the warfarin dose. Working with Rieder et al., we validated novel noncoding SNPs in the vitamin K epoxide reductase. Methods: After PCR amplification, we used Pyrosequencing® to genotype DNA regions for 2 coding CYP2C9 SNPs, *2 (C430T) and *3 (A1075C), and for 4 noncoding VKORC1 SNPs: C861A, T5808G, G6853C, and G9041A. A total of 368 patients were studied in the Washington University cohort and complete results are available for 340 patients; 28 patients either had missing information or refused permission for their data to be included in this summary. PHASE inferred haplotypes based on VKORC1 genotype at 6853 (chr:31012364): G/G at 6853 was classified as the BB diplotype, G/C as the AB diplotype, and C/C as the AA diplotype. Results: The mean therapeutic dose of warfarin differed significantly among the three haplotype group combinations: 2.7-3.4 mg per day for A/A, 4.3-4.9 mg per day for A/B, and 6.0-6.2 mg per day for B/B (P<0.001). The G6853C SNP and associated haplotype explained 20%-25% of the variability in therapeutic warfarin dose. Conclusions: VKORC1 haplotypes can be used to stratify patients into low-, intermediate-, and high-dose warfarin groups.
Categories of Pharmacogenetic Knowledge
PD Pharmacodynamics and Drug Response
PK Pharmacokinetics
Submitted by PBAT in Submission PS204852.

Figure 14.7.3 Example of PharmGKB phenotype data.

individual phenotype data, such as gender, race, age, dose etc. The Individualized data tab allows the user to view individualized subject data after the user logs in.

6. Click on the Pathways tab to view all pathways associated with VKORC1. Click on the Warfarin Pathway (PD) link to view the simplified diagram of the target of warfarin action and downstream genes and effects.

   See Support Protocol 2 for details.

7. To find drugs and diseases associated with VKORC1, click on the Curated Publications tab to see the manually curated literature information. Under the Details column, click on View to see the evidence of relationship between the drug (warfarin) and gene (VKORC1).

8. Click on warfarin under the Drug column in the Related Drugs from Literature section to bring up the drug page for warfarin, where the detailed pharmacology, mechanism of action, and therapeutic use of the drug are listed in the detail section of the page.

   Both the drug page and disease page follow a design similar to the gene page.

9. Click on Atrial Fibrillation under the Disease column in the Related Diseases from Literature section to see the disease page for Atrial Fibrillation.

10. To download genotype and phenotype data related to VKORC1, click on Downloads/Cross-references tab on the gene page.

    All individualized primary data at PharmGKB are available for download by registered users. For bulk download of some, or all, of the data in PharmGKB for further analysis, please use our SOAP-based Web services (See Support Protocol 4 for details).

11. Under the Downloads/Cross-references tab, click on links to go to external databases where additional information on the VKORC1 gene may be found.

PharmGKB has established bidirectional links with leading gene, protein, and drug resources, such as NCBI Entrez Gene (Maglott et al., 2007), GeneCards (Safran et al., 2002), UniProtKB (Wu et al., 2006), and DrugBank (Wishart et al., 2006). We also provide links from the gene page to Online Mendelian Inheritance in Man (OMIM; Hamosh, et al., 2005), the Genome Data Base (GDB; Letovsky et al., 1998), NCBI RefSeq sequences (Pruitt et al., 2007), and their associated Gene Ontology annotations (Harris et al., 2004). In the Common Searches section immediately below the Cross-references, users can check to see if their gene of interest is part of any pathway documented within public pathway databases, such as the Kyoto Encyclopedia of Genes and Genomes (KEGG; Kanehisa et al., 2004), BioCarta (http://www.biocarta.com), or Reactome (UNIT 8.7; Joshi-Tope et al., 2005).

SUPPORT PROTOCOL 1

ORIENTATION TO THE PharmGKB VARIANT PAGE

The PharmGKB gene variant page contains a variant browser and a variant table. The variant browser displays all the polymorphisms in the gene of interest documented within various sources, such as PharmGKB primary data, single-nucleotide polymorphism (SNP) arrays (*http://www.illumina.com* and *http://www.affymetrix.com*), NCBI Single Nucleotide Polymorphism Database (dbSNP; Sherry et al., 2001), and Japanese Single Nucleotide Polymorphisms Database (jSNP; Hirakawa et al., 2002). The variant table below the browser lists detailed nonarray genotype data in PharmGKB, such as their genomic positions, functional annotation for variants of interests, structural view of the coding variants, polymorphism frequencies, and assay types. Both the variant table and PharmGKB SNP array data for the gene of interest are available for download at the bottom of the variant page.

Necessary Resources

Hardware

Computer with an internet connection

Software

Any up-to-date browser will work

Files

No input files required

1. Open the PharmGKB homepage at *http://www.pharmgkb.org* in a Web browser and click on the genotyped genes icon in the browse section. This will lead to the page containing all genes with genotype information listed in alphabetical order.
2. Click on the letter V to go to all genes starting with the letter V. The number 4 to the right of the letter V indicates the number of genes with variant data starting with that specific letter.
3. Click on the "[variants]" link to the right of VKORC1 to go to the variant gene page for VKORC1.

The variant gene page contains a variant browser at the top and a variant table below (Fig. 14.7.4).

The variant browser gives a graphical representation of gene structure and location of variants contained within PharmGKB (including those derived from whole genome SNP arrays). Variants collected from external SNP databases, such as dbSNP and jSNP are also available through the variant browser, allowing users to easily compare and contrast SNPs

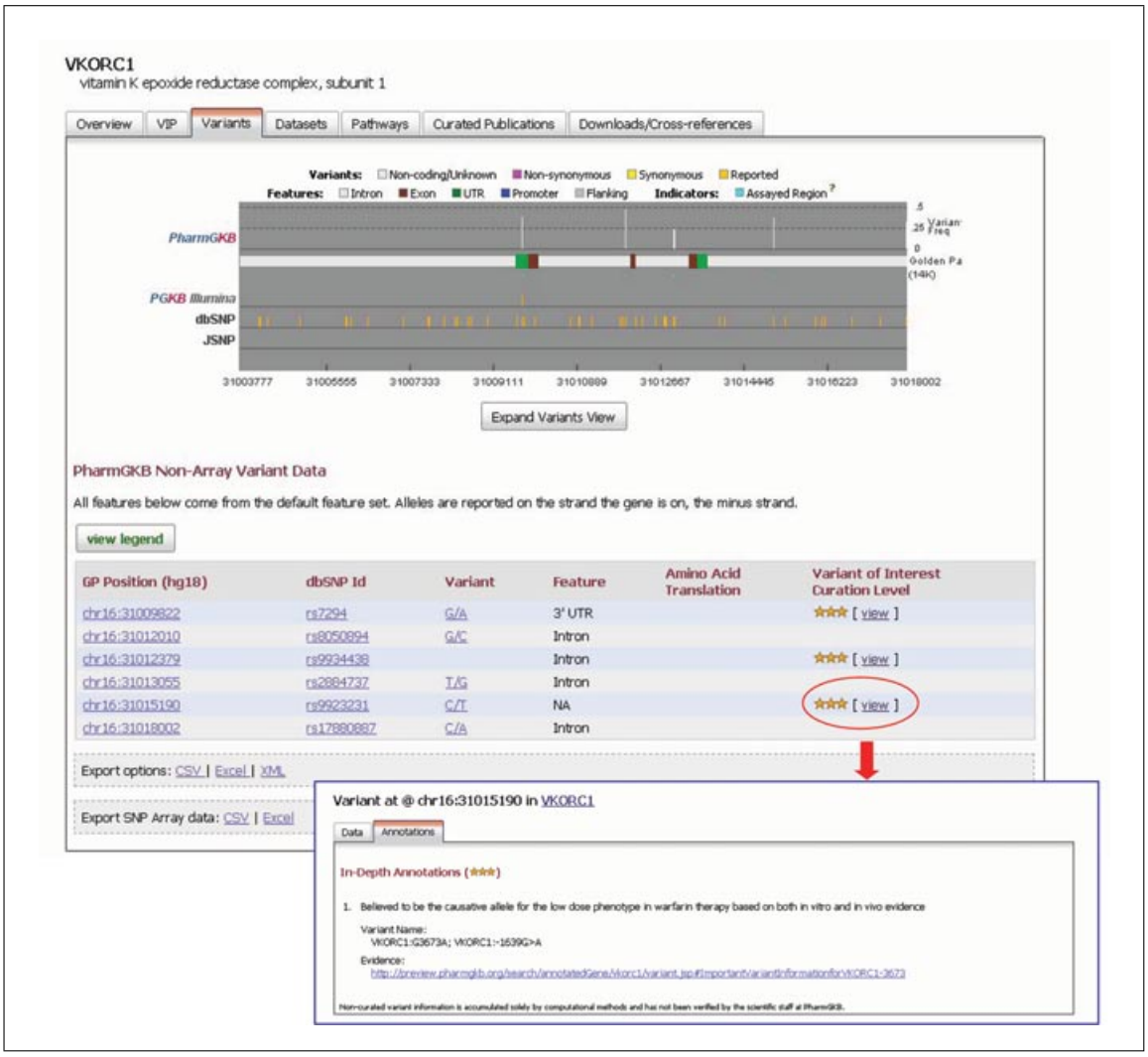


Figure 14.7.4 Example of PharmGKB Variant Gene Page (VKORC1) with variant browser on the top and variant table below.

from different resources and identify regions that have a high density of polymorphisms. Each tick on the browser represents a variant from the respective resource. The gene features are also color-coded to differentiate exons, introns, promoters, and untranslated regions (UTRs). By using the magnification and move tools below the browser, the user can move or zoom into the specific region of interest for a gene.

4. Scroll down the variant page to locate the variant table below the browser to see PharmGKB nonarray variants and their genomic positions, functional annotations, frequencies, and assay types.

   Clicking on the link under the GP Position column will open the UCSC Genome Browser (UNIT 1.4; Kuhn et al., 2007) in another window. Clicking on the link under the "dbSNP Id" column will open the dbSNP entry that corresponds to the variant in another window (UNIT 1.3). The entries in the Feature and Amino Acid Translation columns are derived from the default reference sequence from NCBI for the specific gene.

5. Click on the G/A variant (at GP position chr16:31009822) in the variant column. This will display the variant report that includes the reference sequence for the specific variant.

6. Click on the "stars" under the Variants of Interest Curation Level column to see the brief functional summary for the variants and their literature support evidence.

Stars are used to indicate the level of annotation applied to the variants: 1 star for noncurated annotations, 2 stars for curated annotations, and 3 stars for in-depth annotations. Note that noncurated variant information is accumulated solely by computational methods and has not been verified by the scientific staff at PharmGKB.

7. Click on the Expand Variants View button, below the variant browser, to see the full variant table containing more in-depth information collected for the variants, such as frequencies and assay types. Click on the link in the Frequency column (e.g., "58.97%/41.03%" at GP Position chr16:31009822) to see a breakdown of the frequency across all variants reported by racial categories.

   The value in the Frequency column is calculated by aggregating all variants reported at that Golden Path position. Clicking on the value will allow the user to drill down further for frequency data by race or ethnicity. The entry in the Number of Chromosomes column is typically twice the number of subjects that were in the submitted sample set. Each variant is also annotated with the detailed genotyping assay performed on that variant in the Assay Types column.The Phi link (Φ) in the Flags column indicates that phenotype data was collected on subjects genotyped for that Golden Path position.

8. Click on the View link under the Data column in the full variant table to see subject genotype data reported at the specific position. (Individual level genotype data requires user registration).

9. Scroll down to the bottom of the variant page to export the variant table in CVS/Excel/XML formats, along with any SNP array data from PharmGKB, for the gene of interest.

SUPPORT PROTOCOL 2

ORIENTATION TO THE PharmGKB PATHWAY PAGES

The interactive drug-centered pathways displayed in PharmGKB provide an overview of how genes are involved in the pharmacokinetics (PK) and pharmacodynamics (PD) of drugs. The pathway diagrams use standard shapes and colors to represent genes, metabolites, drugs, and interactions. All genes and drugs on the pathway diagram are clickable. If the user clicks on these objects, the PharmGKB gene or drug page opens in a new browser window. Below the pathway picture is a description of the pathway that describes the complex gene-drug relationships depicted in the pathway diagram. The pathway authors and the date of the most recent update are listed below the text of the description on the bottom of the pathway diagram. There is a section of useful links and downloads to the right of the pathway picture. At the top right, there is a link to return to the list of pathways. If PharmGKB has both PK and PD pathways for a given drug, the user will see a box with a drop-down menu, allowing them to toggle between the PK and PD pathways. Also listed are links to related drugs, genes, and pathways that have been selected by the authors as being of potential interest to the user. Finally, on the lower right of the pathway page, there are download links for the evidence spreadsheet and the pathway image. The evidence spreadsheet describes each interaction depicted on the pathway, and it includes at least one peer-reviewed article with their PubMed reference identifier in support of each interaction.

Necessary Resources

Hardware

Computer with an internet connection

Software

Any up-to-date browser will work

Files

No input files required

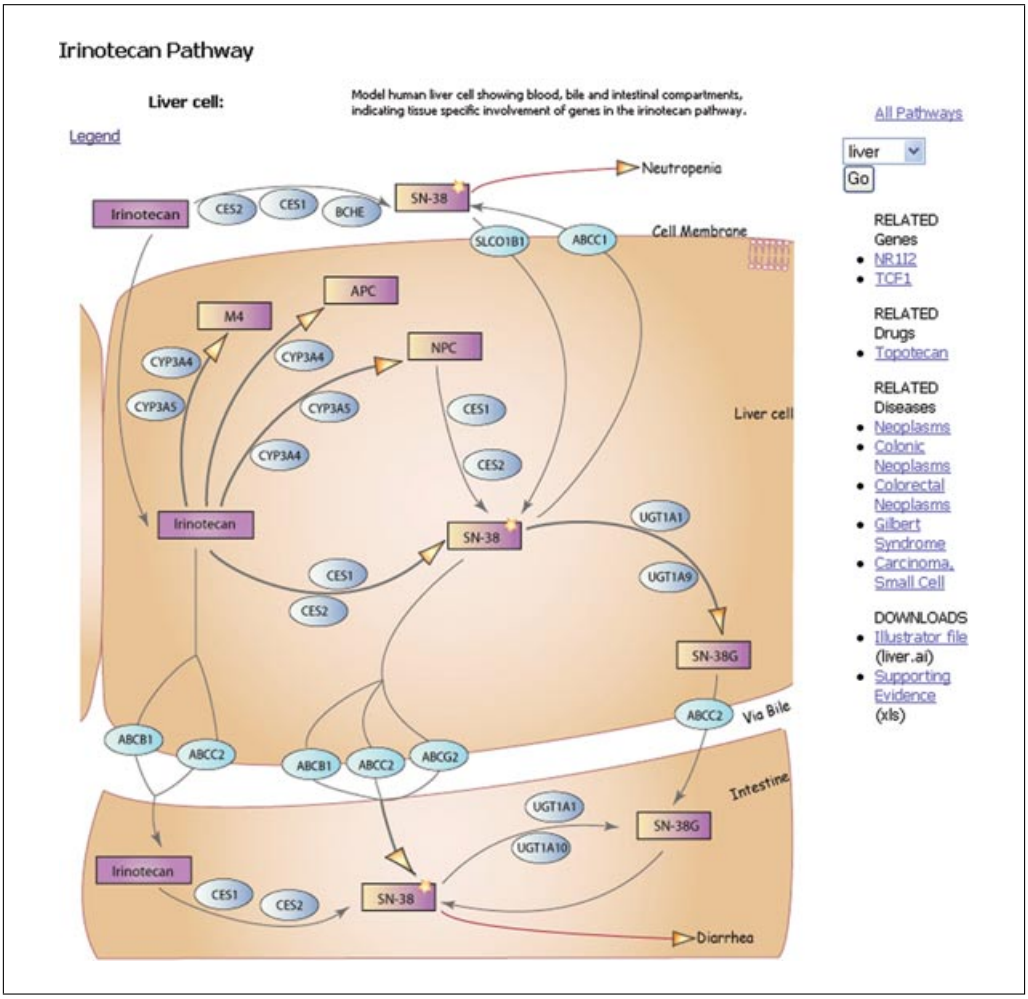


Figure 14.7.5 Example of PharmGKB pathway (Irinotecan pathway). For color version of this figure see *http://www.currentprotocols.com*.

1. Open the PharmGKB homepage at *http://www.pharmgkb.org* in a Web browser and click on the pathway icon to access the list of pathways on PharmGKB. Pathways can also be accessed by clicking on the Search tab, then on Pathways.

2. Jump to page 2 of the pathway list and click on the Irinotecan Pathway link to go to Irinotecan Pathway (Fig. 14.7.5).

   The Irinotecan Pathway shows the pharmacokinetic (PK) process of the chemotherapy drug irinotecan. Genes involved in biotransformation and transport of irinotecan are highlighted in this PK pathway. A pathway that describes the pharmacodynamic (PD) aspect of irinotecan is also available on PharmGKB titled Irinotecan Pathway (Cancer). How irinotecan acts on its target topoisomerase I (TOP1) in the cancer cell is illustrated in this PD pathway.

3. Click on the Legend link on the top-left corner of the pathway diagram to display the standard shapes used to designate the different objects in the pathway.

4. Click on the ABCC1 oval to go to the ABCC1 gene page.

5. Clicking on "irinotecan" will open a pop-up window of the drug page for irinotecan. Click on metabolite SN38 to open a pop-up window that shows the chemical conversion from irinotecan to SN38, it also includes a link to the original article describing the conversion, as well as a link back to the irinotecan drug page.

6. Click on the golden arrow between two objects (SN38 ↔ SN38G) to see a link to the primary data, titled Irinotecan Clinical Data, that support the relationship. This pop-up window also provides a link to the original article describing the influence of UGT1A1 genotype on the rate of glucuronidation of SN38 (PMID:12464801).
7. Click on the pull-down menu in the upper right-hand corner of the pathway page to select an alternative view of the pathway (liver or cancer for Irinotecan pathway, PK or PD in many other pathways).
8. Click on the Illustrator file to download the pathway image in PDF format.

   The original pathway diagram is drawn using Adobe Illustrator. A PDF version of the pathway is available for download to users.

9. Click on Supporting Evidence to download the evidence spreadsheet containing detailed literature support evidence for each step of the pathway.

SUPPORT PROTOCOL 3

ORIENTATION TO THE VIP GENE PAGE

Very Important Pharmacogenes (VIPs) are structured summaries containing key information for genes that are important for pharmacokinetic or pharmacodynamic effects of drugs. These in-depth annotated summaries include information on important variants of the gene, mapping information, haplotypes, population frequencies, phenotypes, and interacting drugs. Supporting key PubMed references are included in each gene summary. These annotations are manually curated.

Necessary Resources

Hardware

Computer with an internet connection

Software

Any up-to-date browser will work

Files

No input files required

1. Open the PharmGKB homepage at *http://www.pharmgkb.org* in a Web browser and click the VIP gene icon. This will lead to the page for all VIPs within PharmGKB, in alphabetical order.
2. Click on View under the VIP Page column to go to the ABCB1 VIP gene summary.

   VIP pages can also be accessed from a gene page by clicking on the VIP tab.

   On the ABCB1 VIP main page, one can find the gene name/symbol, summary, key PubMed IDs, associated pathways and drugs, and important haplotype information (Fig. 14.7.6).

   The VIP gene page itself is constructed from a standard template. The list of contributors to the pathway is provided at the top, followed by links to any important variants, haplotypes, or splice variants that are associated with that gene. Below this information is the VIP summary. Included with every VIP summary are the HGNC gene name, common names or synonyms that frequently appear in the literature for this gene, an introductory paragraph, and key PubMed ID numbers that are associated with the information in the introductory paragraph. If applicable, the VIP page also contains links to PharmGKB pages for the drugs that this gene interacts with, the PharmGKB pathways that the gene appears in, and any phenotypes or diseases for which information is available. At the bottom of the main VIP page, there are links to the important variants, haplotypes, or splice variants that are associated with this gene. In order for a gene to qualify as a VIP gene candidate, it must have at least one variant of pharmacogenomic significance.

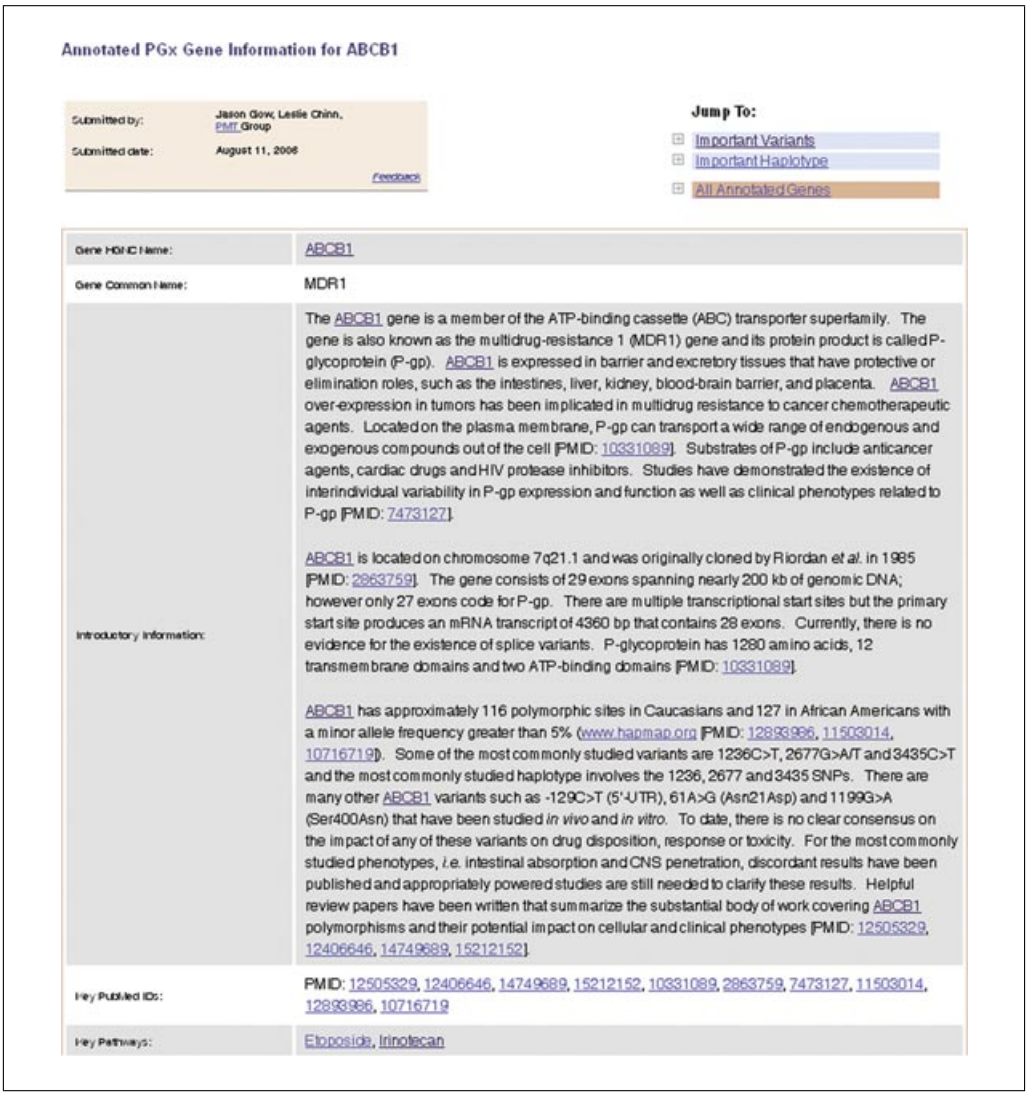
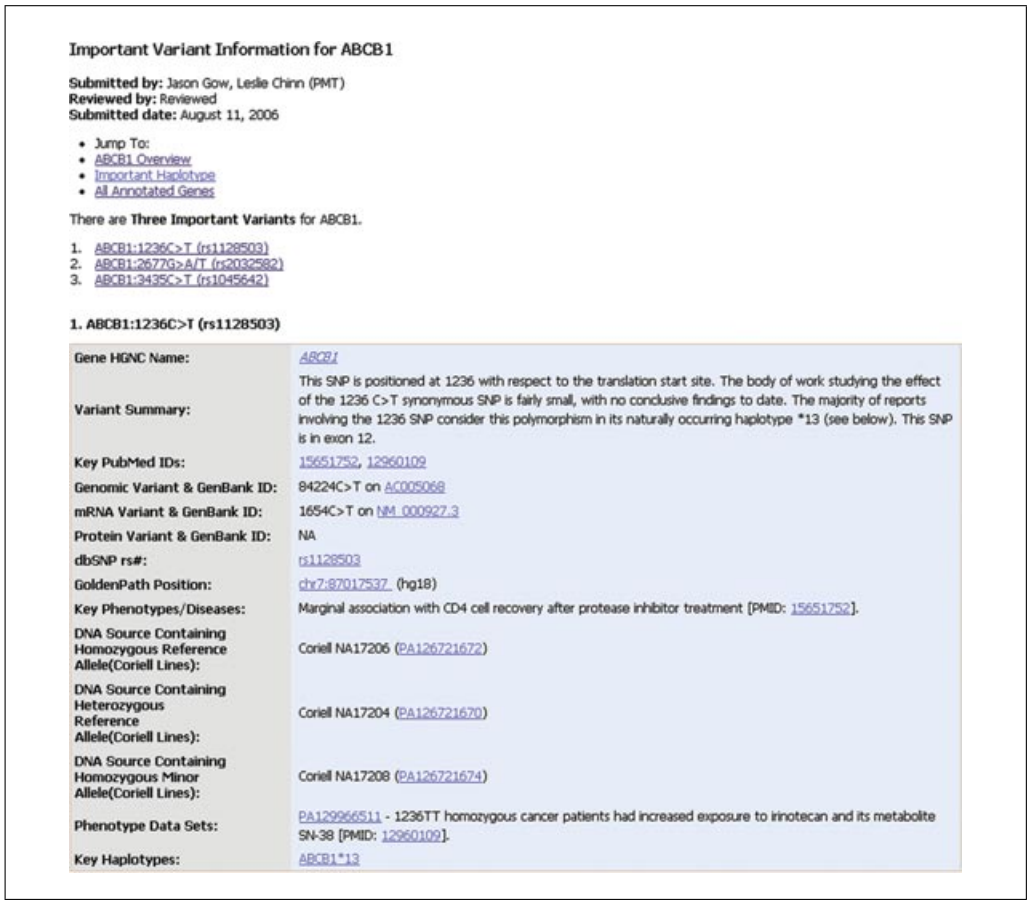

Annotated PGx Gene Information for ABCB1
Submitted by:
Jason Glow, Leslie Chinn, PMT Group
Submitted date:
August 11, 2006
Feedback
Jump To:
Important Variants
Important Haplotype
All Annotated Genes
Gene HGNC Name:
ABCB1
Gene Common Name:
MDR1
Introductory Information:
The ABCB1 gene is a member of the ATP-binding cassette (ABC) transporter superfamily. The gene is also known as the multidrug-resistance 1 (MDR1) gene and its protein product is called P-glycoprotein (P-gp). ABCB1 is expressed in barrier and excretory tissues that have protective or elimination roles, such as the intestines, liver, kidney, blood-brain barrier, and placenta. ABCB1 over-expression in tumors has been implicated in multidrug resistance to cancer chemotherapeutic agents. Located on the plasma membrane, P-gp can transport a wide range of endogenous and exogenous compounds out of the cell [PMID: 10331089]. Substrates of P-gp include anticancer agents, cardiac drugs and HIV protease inhibitors. Studies have demonstrated the existence of interindividual variability in P-gp expression and function as well as clinical phenotypes related to P-gp [PMID: 7473127].
ABCB1 is located on chromosome 7q21.1 and was originally cloned by Riordan et al. in 1985 [PMID: 2863759]. The gene consists of 29 exons spanning nearly 200 kb of genomic DNA; however only 27 exons code for P-gp. There are multiple transcriptional start sites but the primary start site produces an mRNA transcript of 4360 bp that contains 28 exons. Currently, there is no evidence for the existence of splice variants. P-glycoprotein has 1280 amino acids, 12 transmembrane domains and two ATP-binding domains [PMID: 10331089].
ABCB1 has approximately 116 polymorphic sites in Caucasians and 127 in African Americans with a minor allele frequency greater than 5% (www.hapmap.org [PMID: 12893986, 11503014, 10716719]). Some of the most commonly studied variants are 1236C>T, 2677G>A/T and 3435C>T and the most commonly studied haplotype involves the 1236, 2677 and 3435 SNPs. There are many other ABCB1 variants such as -129C>T (5'-UTR), 61A>G (Asn21Asp) and 1199G>A (Ser400Asn) that have been studied in vivo and in vitro. To date, there is no clear consensus on the impact of any of these variants on drug disposition, response or toxicity. For the most commonly studied phenotypes, i.e. intestinal absorption and CNS penetration, discordant results have been published and appropriately powered studies are still needed to clarify these results. Helpful review papers have been written that summarize the substantial body of work covering ABCB1 polymorphisms and their potential impact on cellular and clinical phenotypes [PMID: 12505329, 12406646, 14749689, 15212152].
Key PubMed IDs:
PMID: 12505329, 12406646, 14749689, 15212152, 10331089, 2863759, 7473127, 11503014, 12893986, 10716719
Key Pathways:
Etoposide, Irinotecan

Figure 14.7.6 Example of VIP gene page (ABCB1).

3. Click on the Important Variants link on the top left-hand corner of the VIP gene page to go to the page with detailed information on important variants, external references, and their impact on drug responses (Fig. 14.7.7).

   The VIP variant page is structured similarly to the main VIP page. The top of the VIP variant page contains the list of authors and links back to the main VIP summary, as well as any important haplotypes associated with this gene. Following below is a list showing how many important variants there are for each gene (e.g., there are three important variants for ABCB1). Each important variant has its own entry. This entry contains the HGNC name for the gene, a variant summary that is specific to that particular variant (in contrast to the general summary on the VIP gene page), key PubMed IDs that are associated with the variant summary, complete mapping information for the variants, links to relevant PharmGKB pages for the drugs, and links to phenotype datasets for the gene. The mapping information includes genomic position and accession number, a dbSNP unique identifier (number starts with rs), and its Golden Path position. If applicable, an mRNA and protein position and accession number are also provided. Variant pages may also contain allele frequency tables that list a brief description of the population that has been studied, the number of subjects in that population, the allele frequency of the variant in question, and a link with the PMID number that opens a new browser window with that PubMed abstract. If the variant is part of a haplotype, then there is also a link included at the bottom of the page linking the user to the haplotype that contains the variant.



Important Variant Information for ABCB1

Submitted by: Jason Gow, Leslie Chinn (PMT)
Reviewed by: Reviewed
Submitted date: August 11, 2006

- Jump To:
- ABCB1 Overview
- Important Haplotype
- All Annotated Genes

There are **Three Important Variants** for ABCB1.

1. ABCB1:1236C>T (rs1128503)
2. ABCB1:2677G>A/T (rs2032582)
3. ABCB1:3435C>T (rs1045642)

1. ABCB1:1236C>T (rs1128503)

Gene HGNC Name:	*ABCB1*
Variant Summary:	This SNP is positioned at 1236 with respect to the translation start site. The body of work studying the effect of the 1236 C>T synonymous SNP is fairly small, with no conclusive findings to date. The majority of reports involving the 1236 SNP consider this polymorphism in its naturally occurring haplotype *13 (see below). This SNP is in exon 12.
Key PubMed IDs:	15651752, 12960109
Genomic Variant & GenBank ID:	84224C>T on AC005068
mRNA Variant & GenBank ID:	1654C>T on NM_000927.3
Protein Variant & GenBank ID:	NA
dbSNP rs#:	rs1128503
GoldenPath Position:	chr7:87017537 (hg18)
Key Phenotypes/Diseases:	Marginal association with CD4 cell recovery after protease inhibitor treatment [PMID: 15651752].
DNA Source Containing Homozygous Reference Allele(Coriell Lines):	Coriell NA17206 (PA126721672)
DNA Source Containing Heterozygous Reference Allele(Coriell Lines):	Coriell NA17204 (PA126721670)
DNA Source Containing Homozygous Minor Allele(Coriell Lines):	Coriell NA17208 (PA126721674)
Phenotype Data Sets:	PA129966511 - 1236TT homozygous cancer patients had increased exposure to irinotecan and its metabolite SN-38 [PMID: 12960109].
Key Haplotypes:	ABCB1*13

Figure 14.7.7 Example of important variant page for VIP genes (ABCB1).

4. Click on the Important Haplotype link on the top left-hand corner of the VIP page. This will bring you to a page containing detailed information on known haplotypes for gene of interest, related SNPs, and associated phenotype data files, and their impact on drug responses.

   Haplotype pages are similar to the variant pages, and contain much of the same information. The differences are that there is no mapping information for haplotypes, and these pages also describe how many SNPs contribute to the formation of these haplotypes. A haplotype may be defined by only one SNP, as is the case with many of the CYP haplotypes. In this case, information on the haplotype page may be duplicated on the variant page and vice versa. A separate variant page for any CYP haplotype is included so that the mapping information for that position can also be incorporated. A haplotype page also contains a definitive publication or link to an external Web site, which will take the user to the source that was used to name the haplotype.

SUPPORT PROTOCOL 4

ORIENTATION TO PharmGKB WEB SERVICES

PharmGKB Web services enable our users to download a selected subset of data from PharmGKB via a Simple Object Access Protocol (SOAP) interface. Application programming interface (API) documentation and sample codes are available for any user who wishes to access portions of the database at *http://www.pharmgkb.org/home/projects/webservices/index.jsp*.

Necessary Resources

Hardware

Computer with an internet connection

Software

A user may create a Web services client program in any language of choice. PharmGKB provides Perl and Python clients to access Web services; detailed documentation is available at *http://www.pharmgkb.org/home/projects/webservices/index.jsp*.

Files

Sample codes (Perl and Python) are available for download to access genes, drugs, diseases, and variants using PharmGKB accession numbers. Documentation is found at *http://www.pharmgkb.org/home/projects/webservices/README-perl.txt* or, *http://www.pharmgkb.org/home/projects/webservices/README-python.txt*

1. To use the Perl scripts, download and install the SOAP::lite module from *http://soaplite.com/* or *http://sourceforge.net/projects/soaplite* (available from CPAN)

   API documentation: *http://cpan.uwinnipeg.ca/htdocs/SOAP-Lite/SOAP/Lite.html*

   ```
   % cpan
   cpan> install MIME::Parser
   cpan> install SOAP::Lite
   ```

2. Alternatively, if the user is more familiar with writing or running Python scripts, download and install SOAPpy from:
   http://sourceforge.net/project/showfiles.php?group_id = 26590

   in addition to the python module "fpconst" available from:

   http://cheeseshop.python.org/packages/source/f/fpconst/fpconst-0.7.2.tar.gz

   ```
   % cd fpconst-0.7.2
   % python setup.py install
   ```

Table 14.7.2 Codes for Use with `specialSearch.pl`

Code	Data Type
0	Genes with pharmacokinetic (PK) significance
1	Genes with pharmacodynamic (PD) significance
2	Genes with PharmGKB variant data
3	Genes with PK variants
4	Genes with PD variants
5	Drugs with supporting information
6	Diseases with supporting information
7	Phenotype datasets
8	Pathways with PGx significance
9	Annotated publications describing relationships between genes, drugs, and diseases
10	Literature annotations, pathways, and phenotype datasets annotated with a pharmacokinetics (PK) COE
11	Literature annotations, pathways, and phenotype datasets annotated with a pharmacodynamics (PD) COE
12	Literature annotations and phenotype datasets annotated with a clinical outcome (CO) COE

```
Then, install the SOAPpy module itself
   % cd SOAPpy-0.12.0
   % python setup.py install
```

3. If Perl client is used, simple client programs are available to access. Web services on PharmGKB (see *https://www.pharmgkb.org/home/projects/webservices/README-perl.txt* for details). For example, users can use `specialSearch.pl <searchType(integer)>` to access various types of data from PharmGKB (refer to Table 14.7.2 for special codes for searchTypes). Typing `% perl specialSearch.pl 0` will output all genes with pharmacokinetic relevance at PharmGKB.

COMMENTARY

Background Information

PharmGKB began as the central data repository for the Pharmacogenetics Research Network (PGRN) and scientific community at large in 2000 (Giacomini et al., 2007; Long, 2007). It is designed to be a publicly available knowledge base with scientifically documented information connecting phenotypes to genotypes. Over the past 8 years of development (funded by NIH), PharmGKB has grown to be an integrated resource that provides data on variants in genes, their relationship to drug response phenotype, the phenotype data, and curated knowledge in the forms of drug centered pathways, pharmacogene summaries (VIPs), and literature annotations (Hodge et al., 2007). PharmGKB currently houses variant data associated with >600 genes, >2000 manually curated literature annotations, 52 drug-centered pathways, and 27 VIP gene summaries. Our comprehensive content makes it easier and faster for our users to access key pharmacogenomic information without repeating searches in multiple databases.

PharmGKB primary data comprises both genotype data and phenotype data. Initially, the data depository was seeded by data from the PGRN and mainly focused on a handful of genes at a time. With the rapid advancement and widespread use of high-throughput technology to measure gene variation and gene expression, the field of pharmacogenomics has evolved to explore a much larger set of genes, up to the whole genome. This also includes how variations in these larger gene sets work in concert to affect drug response. PharmGKB has expanded its capacity to accommodate large-scale high-throughput data, which may involve a large number of samples assayed across the entire genome. SNP array data can now be viewed and downloaded from the PharmGKB. PharmGKB also houses large data submissions from beyond PGRN. In 2006, Applied Biosystems posted genotype data for >220 drug response genes from four human populations (i.e., Caucasian, African American, Chinese, and Japanese) on PharmGKB. Allele frequencies for each of these populations were calculated and are available from the variant frequency report. PharmGKB is also the central repository for the International Warfarin Pharmacogenetics Consortium (IWPC; *http://www.pharmgkb.org/views/project.jsp?pId = 56*). The goal of this consortium is to create a merged international dataset (including >5700 patients) in order to develop the best strategy for predicting the therapeutic dose of warfarin.

In addition to our data mission, PharmGKB is curating pharmacogenomic knowledge, including summarizing drug-centered pathways, annotating very important pharmacogenes (VIPs) and primary literature. Unlike other pathway resources—e.g., KEGG (*UNIT 1.12*), Reactome (*UNIT 8.7*), Biocarta, GenMAPP (*UNIT 7.5*)—that primarily focus on physiological processes, PharmGKB is the only resource that focuses on drug-centered pathways, particularly pharmacokinetic (PK) pathways. This effort is valuable to the scientific community as our pathways enable researchers to conduct in-depth analysis of various forms of experimental data within the framework of curated drug response pathways. Our pharmacokinetics (PK) pathways describe candidate genes involved in the absorption, distribution, metabolism, and excretion of a given drug, while the pharmacodynamic (PD) pathways illustrate the physiological effects of the drug, its mechanism of action, and possible side effects. Currently, there are 39 interactive drug-centered pathways created in collaboration with experts in the pharmacogenomic area. PharmGKB pathways have been

widely quoted by the scientific community for their unique content (Mangravite et al., 2006; Scripture and Figg, 2006). The VIP gene summaries are another unique knowledge-rich feature provided by PharmGKB for key genes that are involved in modulating drug response. Each VIP summary is constructed using a structured template and includes detailed information about a given gene, including its important polymorphisms, haplotypes, phenotypes, and complete mapping information. An allele frequency table may also be included if the specific variant is studied extensively in different populations. VIP summaries are encyclopedia-like encapsulations for genes that require tremendous amounts of manual curation. They can potentially save scientists countless hours of time in their own literature mining process, which can be tedious, repetitive, and time consuming. To keep our pathways and VIPs current, PharmGKB updates them every 2 years to incorporate any new interactions or correct any erroneous information that is being displayed.

PharmGKB provides a wealth of information to facilitate the design of a pharmacogenetics study, such as identifying genetic markers for a patient's response to a therapeutic agent. A scientist designing the study can use PharmGKB in the following manner to pick the best candidates genes and variants from our integrated knowledge base.

Identify candidate genes important for pharmacokinetics or pharmacodynamics of the drug used in the study

If the pathway for the specific drug is available through PharmGKB, this is the best place to start looking for the candidate genes. The genes on our drug-centered pathway are known to be involved in the disposition or mechanism of action of the drug, and the user can click on each gene in the pathway to delve down to the detailed variant information associated with that gene. If no pathway is currently available for the drug of interest, the scientist can first perform a search for the drug in PharmGKB, open the drug page, and then go to the section on Related Genes From Literature to find candidate genes that are implicated in drug response, as well as their literature evidence to decide on which genes to choose for the study.

Find functional variants for the candidate genes chosen

If there is a PharmGKB VIP page available for the gene, the VIP will identify the important variants and haplotypes for the gene of interest. Alternatively, the scientist can go to the PharmGKB variant page and browse through the variant table, which lists all the variants for the gene, their genomic position, functional role, frequency, and assay type. SNPs that reside in the exon or promoter regions of the gene, and SNPs that lead to changed amino acid composition, inactive protein, or changed expression of the gene are good candidates to be included for the study. Annotations for variants that have been studied for phenotypic consequences are tagged with the star system as discussed in Support Protocol 1.

Determine if the population frequencies for the chosen variants are desirable

This step will further screen out SNPs that may be too rare in the population that will be included in the study. The frequency information can be found in the frequency column on the PharmGKB variant table. Clicking on the frequency value displays the breakdown of frequencies by racial categories.

Find assay and primer information for the chosen variants

Clicking on the nucleotide changes in the Variant column of the PharmGKB variant table will allow the user to find information such as assay methods and primers. For instance, if the Taqman assay was used to genotype a specific drug metabolizing enzyme variant, PharmGKB provides a direct link to ordering information at Applied Biosystems to help the user identify the material required for the study. By iterating through these steps, a scientist can compile a short list of candidate genes and SNPs that can be used in a study to identify genetic markers that might explain and predict the efficacy and adverse effect profiles of the drug of interest.

Pharmacogenomics is a rapidly evolving field with many unmet challenges in translating the scientific findings in pharmacogenomics to clinical practice. However, the increasing understanding of how a person's genetic makeup can influence his or her response to drugs provides the opportunity to improve the drug development process and provide more effective and safer therapies for individual patients. PharmGKB will continue its efforts to aggregate, integrate, and annotate the latest findings in pharmacogenomic research, and provide tools and context to catalyze scientific discoveries.

Critical Parameters and Troubleshooting

PharmGKB is designed to be a valuable resource for both expert researchers in the pharmacogenomics field, as well as for novice users and the general public. PharmGKB's homepage prominently displays the information that our users are looking for most frequently with a distinct icon system to represent different data types and knowledge. Typical searches conducted at PharmGKB are for information about drugs, genes, diseases, and pathways. Searches can be conducted using the Web-search engine-like search box. If too many search results are returned, users can narrow the search with more specific terms. Alternatively, a user can use our Search tab and simple query to limit the domain of the search to a specific area of interests (e.g., genes with genotype data; the relevant literature on drug X; diseases with PharmGKB primary data). If the user encounters difficulties in finding information of interest, using alternative names, partial names, or a loosening of the search criteria is suggested. Alternatively, if nothing is returned under the "database search" tab, the user should look for results under the Web site Search tab as PharmGKB has full-text indexing to allow users to search across the entire Web site.

We welcome all feedback regarding the PharmGKB. Questions and concerns can be sent to *feedback@pharmgkb.org*. Our scientific staff will respond to your inquiry within 48 hr.

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Exploring Human Metabolites Using the Human Metabolome Database

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ABSTRACT

The Human Metabolome Database (HMDB) is a Web-based bioinformatic/cheminformatic resource with detailed information about human metabolites and metabolic enzymes. It can be used for fields of study including metabolomics, biochemistry, clinical chemistry, biomarker discovery, medicine, nutrition, and general education. In addition to its comprehensive literature-derived data, the HMDB contains an extensive collection of experimental metabolite concentration data for plasma, urine, CSF, and/or other biofluids The HMDB is fully searchable, with many tools for viewing, sorting and extracting metabolite names, chemical structures, biofluid concentrations, enzymes, genes, NMR or MS spectra, and disease information. Each metabolite entry in the HMDB contains an average of 90 separate data fields including a comprehensive compound description, names and synonyms, chemical structure information, physico-chemical data, reference NMR and MS spectra, normal and abnormal biofluid concentrations, tissue locations, disease associations, pathway information, enzyme data, gene sequence data, and SNP and mutation data, as well as extensive links to images, references and other public databases. *Curr. Protoc. Bioinform.* 25:14.8.1-14.8.45. © 2009 by John Wiley & Sons, Inc.

Keywords: Database • metabolomics • bioinformatics • cheminformatics • biochemistry • genomics • proteomics • systems biology • pathways • spectra

INTRODUCTION

The Human Metabolome Database (HMDB) is a unique Web-based bioinformatic/cheminformatic resource with detailed information about human metabolites and the enzymes that metabolize them. It is designed to be used for a variety of applications and fields of study including metabolomics, biochemistry, clinical chemistry, biomarker discovery, medicine, nutrition, and general education. The HMDB currently contains more than 2900 human metabolite entries that are linked to more than 28,000 different synonyms. These metabolites are further connected to some 77 nonredundant pathways, 3364 distinct enzymes, 103,000 SNPs, and 862 metabolic diseases (genetic and acquired). Much of this information was gathered manually or semi-automatically from thousands of books, journal articles, and electronic databases. In addition to its comprehensive literature-derived data, the HMDB contains an extensive collection of experimental metabolite concentration data for plasma, urine, CSF, and/or other biofluids for more than 960 compounds. The HMDB also has about 570 compounds for which experimentally acquired "reference" ^{1}H and ^{13}C NMR and MS/MS spectra have been collected.

The HMDB is fully searchable, with many built-in tools for viewing, sorting and extracting metabolite names, chemical structures, biofluid concentrations, enzymes, genes,

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NMR or MS spectra, and disease information. Each metabolite entry (called a "MetaboCard") in the HMDB contains an average of 90 separate data fields including a comprehensive compound description, names and synonyms, chemical structure information, physico-chemical data, reference NMR and MS spectra, normal and abnormal biofluid concentrations, tissue locations, disease associations, pathway information, enzyme data, gene sequence data, and SNP and mutation data, as well as extensive links to images, references and other public databases including the Kyoto Encyclopedia of Genes and Genomes (KEGG; *UNIT 1.12*; Kanehisa et al., 2004), PubChem (Wheeler et al., 2005), Chemical Entities of Biological Interest (ChEBI; Brooksbank et al., 2005), MetaCyc (*UNIT 1.17*; Krummenacker et al., 2005), Protein Data Bank (PDB; *UNITS 1.9 & 14.3*), SwissProt (Bairoch et al., 2005), and GenBank (Wheeler et al., 2005). In this unit, readers will be shown how to effectively navigate through and retrieve data from the HMDB Web site (Basic Protocol 1), how to perform chemical structure similarity searches (Basic Protocol 2), and how to identify metabolites via spectral matching (Basic Protocol 3). Basic Protocols 2 and 3 take advantage of the HMDB's extensive collections of chemical structures and spectra (NMR, MS, and GC-MS), respectively.

BASIC PROTOCOL 1

NAVIGATING THE HUMAN METABOLOME DATABASE WEB SITE

The Human Metabolome Database (HMDB) can be accessed at: *http://www.hmdb.ca/*. It is compatible with most up-to-date Web browsers as long as they are equipped with a Java interpreter. The HMDB Web site is navigated using hyperlinked menus or text. The appearance and functionality of the HMDB Web site should be the same regardless of the user's browser or operating system. On the home page, and nearly every page of the HMDB Web site, there is a menu bar located at the top of the page. This menu bar contains hyperlinks that allow the user to navigate between specific display or search pages within the Web site. For most searches, text is typed or pasted into standard text boxes and the search function is launched by clicking the mouse pointer on a Search or Submit button. The home page provides a text search box as well as an overview of the database and some of its key features. This protocol describes in detail how to find, view, interpret, and retrieve data from the HMDB Web site.

Necessary Resources

Hardware

Computer with Internet access

Software

An up-to-date Web browser, such as Internet Explorer (*http://www.microsoft.com/ie/*), Firefox (*http://www.mozilla.com/*), Netscape (*http://browser.netscape.com/*), Opera (*http://www.opera.com/*), or Safari (*http://www.apple.com/safari/*). The Web browser must be capable of handling Java applets (i.e., equipped with a recent version of the Java interpreter).

Files

None

Standard MetaboCard Overview

1. With your preferred Web browser, visit the Human Metabolome Database (HMDB) Web site at *http://hmdb.ca/*.

   The HMDB home page (Fig. 14.8.1) has a light gray menu bar located near the top of the page with fourteen clickable links: Home, Browse, Biofluids, Tissues, ChemQuery, TextQuery, SeqSearch, DataExtractor, MS/MS Search, MS Search, GC/MS Search, NMR Search, Download, and Explain. The menu bar allows the user to easily navigate the HMDB's browsing and search utilities. Below the menu bar is a text box next to which

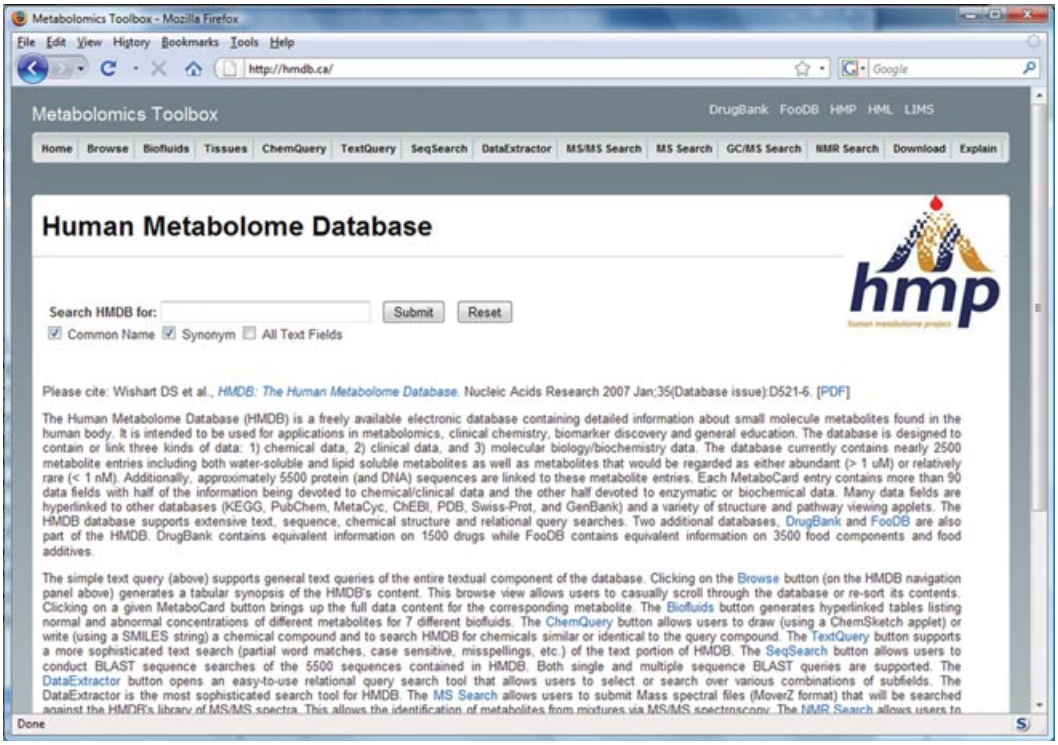

Metabolomics Toolbox - Mozilla Firefox
File Edit View History Bookmarks Tools Help
http://hmdb.ca/
Metabolomics Toolbox
DrugBank FooDB HMP HML LIMS
Home Browse Biofluids Tissues ChemQuery TextQuery SeqSearch DataExtractor MS/MS Search MS Search GC/MS Search NMR Search Download Explain
Human Metabolome Database
Search HMDB for:
Submit
Reset
Common Name Synonym All Text Fields
Please cite: Wishart DS et al., HMDB: The Human Metabolome Database. Nucleic Acids Research 2007 Jan;35(Database issue):D521-6. [PDF]
The Human Metabolome Database (HMDB) is a freely available electronic database containing detailed information about small molecule metabolites found in the human body. It is intended to be used for applications in metabolomics, clinical chemistry, biomarker discovery and general education. The database is designed to contain or link three kinds of data: 1) chemical data, 2) clinical data, and 3) molecular biology/biochemistry data. The database currently contains nearly 2500 metabolite entries including both water-soluble and lipid soluble metabolites as well as metabolites that would be regarded as either abundant (> 1 uM) or relatively rare (< 1 nM). Additionally, approximately 5500 protein (and DNA) sequences are linked to these metabolite entries. Each MetaboCard entry contains more than 90 data fields with half of the information being devoted to chemical/clinical data and the other half devoted to enzymatic or biochemical data. Many data fields are hyperlinked to other databases (KEGG, PubChem, MetaCyc, ChEBI, PDB, Swiss-Prot, and GenBank) and a variety of structure and pathway viewing applets. The HMDB database supports extensive text, sequence, chemical structure and relational query searches. Two additional databases, DrugBank and FooDB are also part of the HMDB. DrugBank contains equivalent information on 1500 drugs while FooDB contains equivalent information on 3500 food components and food additives.
The simple text query (above) supports general text queries of the entire textual component of the database. Clicking on the Browse button (on the HMDB navigation panel above) generates a tabular synopsis of the HMDB's content. This browse view allows users to casually scroll through the database or re-sort its contents. Clicking on a given MetaboCard button brings up the full data content for the corresponding metabolite. The Biofluids button generates hyperlinked tables listing normal and abnormal concentrations of different metabolites for 7 different biofluids. The ChemQuery button allows users to draw (using a ChemSketch applet) or write (using a SMILES string) a chemical compound and to search HMDB for chemicals similar or identical to the query compound. The TextQuery button supports a more sophisticated text search (partial word matches, case sensitive, misspellings, etc.) of the text portion of HMDB. The SeqSearch button allows users to conduct BLAST sequence searches of the 5500 sequences contained in HMDB. Both single and multiple sequence BLAST queries are supported. The DataExtractor button opens an easy-to-use relational query search tool that allows users to select or search over various combinations of subfields. The DataExtractor is the most sophisticated search tool for HMDB. The MS Search allows users to submit Mass spectral files (MoverZ format) that will be searched
Done

Figure 14.8.1 A screen shot of the HMDB home page. At the top of the page is a menu bar (light gray) with fourteen clickable menu choices (in black). This menu bar allows users to take advantage of the HMDB's rich selection of browsing and searching utilities. Below the menu bar, information about how to use, contact, and reference the HMDB are provided.

appear the words "Search HMDB for:". This text search utility is prominently displayed near the top of nearly every HMDB Web page and allows the user to search for all metabolite entries or MetaboCards with matching text. The user can match by three different criteria: common name, synonyms, all text fields, or any combination of the three. Below the text search box is a brief description of the HMDB, how to use its features, and how to reference it.

2. Click in the text search box near the top of the home page to the right of the text "Search HMDB for:"**.** Once the cursor appears, type `histidine` and make sure that Common Name and Synonyms are checked, but not All Text Fields. Click on the Search button. Within a few seconds a four-column table should be displayed with all MetaboCards containing "histidine" within the Common Name and Synonym fields (Fig. 14.8.2). Column one contains the HMDB accession numbers (hyperlinked) while column two displays the common names for all matching human metabolites. Columns three and four display the chemical formulas and molecular weights, respectively.

HMDB text searches are not case-sensitive and support a variety of searches (i.e., complete words, numbers, multiple words, phrases, and partial words). For example, if the user searches the HMDB using the query terms `hi`*,* `his`*,* `hist`*,* `histid`*,* `histidine`*, or* `histidine` *with the common name and synonyms check boxes checked, the number of hits varies from 457 using* `hi` *to 23 using* `his`*, to 19 using* `hist`*. However, searching for the more specific term* `histidine` *returns only 12 hits. If the user performs the same search for* `histidine` *with all three checkboxes checked (i.e., common names, synonyms, and all text fields), the number of hits increases dramatically to 173. In this latter case, by selecting all fields, the user is searching through most text in the database*

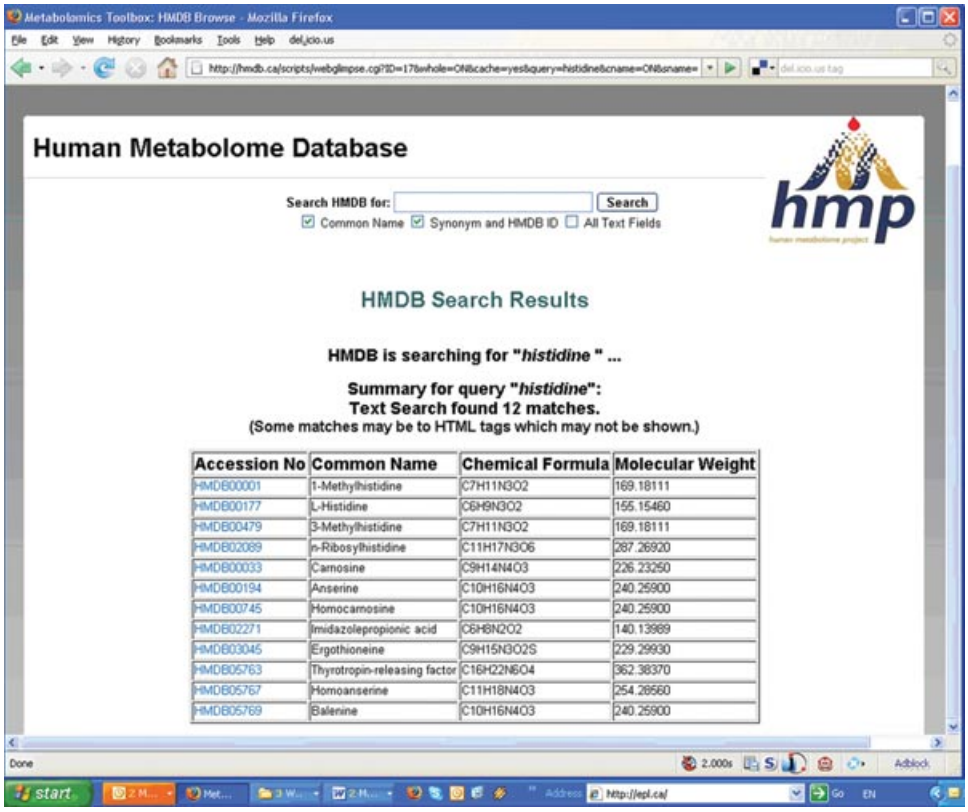

Metabolomics Toolbox: HMDB Browse - Mozilla Firefox
Human Metabolome Database
Search HMDB for:
Search
Common Name
Synonym and HMDB ID
All Text Fields
HMDB Search Results
HMDB is searching for "histidine " ...
Summary for query "histidine":
Text Search found 12 matches.
(Some matches may be to HTML tags which may not be shown.)
Accession No | Common Name | Chemical Formula | Molecular Weight
HMDB00001 | 1-Methylhistidine | C7H11N3O2 | 169.18111
HMDB00177 | L-Histidine | C6H9N3O2 | 155.15460
HMDB00479 | 3-Methylhistidine | C7H11N3O2 | 169.18111
HMDB02089 | n-Ribosylhistidine | C11H17N3O6 | 287.26920
HMDB00033 | Carnosine | C9H14N4O3 | 226.23250
HMDB00194 | Anserine | C10H16N4O3 | 240.25900
HMDB00745 | Homocarnosine | C10H16N4O3 | 240.25900
HMDB02271 | Imidazolepropionic acid | C6H8N2O2 | 140.13989
HMDB03045 | Ergothioneine | C9H15N3O2S | 229.29930
HMDB05763 | Thyrotropin-releasing factor | C16H22N6O4 | 362.38370
HMDB05767 | Homoanserine | C11H18N4O3 | 254.28560
HMDB05769 | Balenine | C10H16N4O3 | 240.25900
Done

Figure 14.8.2 A screen shot showing the HMDB search results for the word `histidine`. The HMDB accession numbers on the left side of the table are hyperlinked. Each accession number corresponds to a human metabolite in the database.

including gene and protein names for metabolic enzymes. It is important to note that the actual sequence text is not searched. The HMDB's text search function is performed using a rapid, index-based query tool called GLIMPSE (Manber and Bigot, 1997). When no hits are found, the text search engine uses a text similarity function to see if the query word has some similarity to a known common name or chemical synonym. For instance, if a user types `hystidine`*, the query engine will return a message with "Sorry, cannot find what you are looking for. Did you mean Histidine?" The proposed compound name is also hyperlinked. Clicking on the hyperlink will launch a text search for* `Histidine`*.*

3. Click on the hyperlinked accession number for 1-Methylhistidine (HMDB00001). A new window should appear containing the "MetaboCard" for 1-methylhistidine (Fig. 14.8.3).

For every metabolite in the Human Metabolome Database, there is one MetaboCard. This design is analogous to the very successful DrugCards concept used in DrugBank (Wishart et al., 2006). Each MetaboCard entry contains more than 90 data fields, with the first half of the information being devoted to chemical and clinical data and the other half devoted to enzymatic or biochemical data (see Table 14.8.1). The MetaboCard information is laid out as follows: (1) metabolite nomenclature; (2) chemical/physical properties; (3) structural data; (4) spectral data; (5) location data (cellular, biofluid, and tissue); (6) concentration data; (7) associated disorders data; (8) pathway data; (9) enzyme data; and (10) SNP data for each metabolizing enzyme. If a metabolite has more than one metabolizing enzyme, the genetic and protein data fields are repeated for each metabolizing enzyme. In addition to providing comprehensive numeric, sequence, and textual data, each MetaboCard also contains hyperlinks to other databases, abstracts, digital images, and interactive applets for viewing molecular structures.

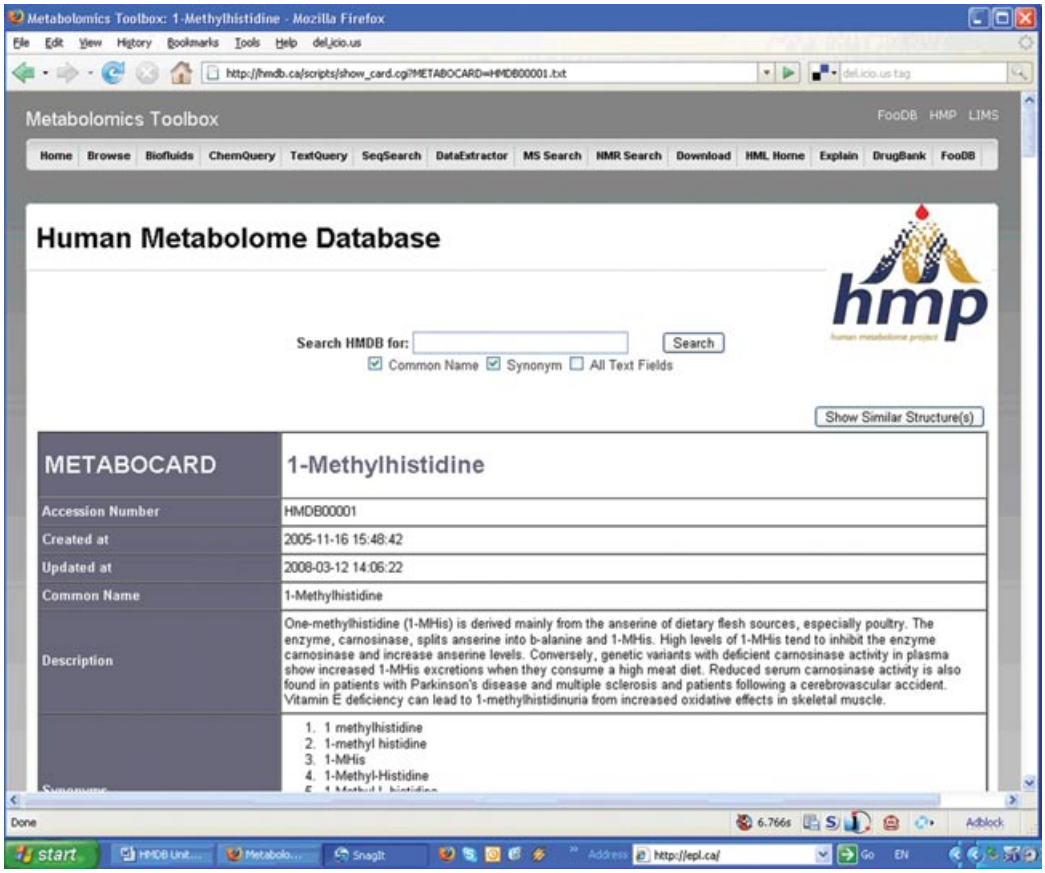

Metabolomics Toolbox: 1-Methylhistidine - Mozilla Firefox
Metabolomics Toolbox
Human Metabolome Database
Search HMDB for:
Search
Common Name
Synonym
All Text Fields
Show Similar Structure(s)
METABOCARD
1-Methylhistidine
Accession Number
HMDB00001
Created at
2005-11-16 15:48:42
Updated at
2008-03-12 14:06:22
Common Name
1-Methylhistidine
Description
One-methylhistidine (1-MHis) is derived mainly from the anserine of dietary flesh sources, especially poultry. The enzyme, carnosinase, splits anserine into b-alanine and 1-MHis. High levels of 1-MHis tend to inhibit the enzyme carnosinase and increase anserine levels. Conversely, genetic variants with deficient carnosinase activity in plasma show increased 1-MHis excretions when they consume a high meat diet. Reduced serum carnosinase activity is also found in patients with Parkinson's disease and multiple sclerosis and patients following a cerebrovascular accident. Vitamin E deficiency can lead to 1-methylhistidinuria from increased oxidative effects in skeletal muscle.
1. 1 methylhistidine
2. 1-methyl histidine
3. 1-MHis
4. 1-Methyl-Histidine

Figure 14.8.3 A screen shot of the MetaboCard for 1-methylhistidine.

4. To survey the type of information that is displayed in a typical MetaboCard, use the scroll bar on the right side of your browser's window to scroll down the 1-methylhistidine MetaboCard page. Basically the MetaboCard consists of two columns, a left column shaded in gray and a right column shaded in white. The gray column contains the field names while the white column contains metabolite-specific information. The upper portion of each MetaboCard provides detailed information about the names, synonyms, chemical structure, and other physical-chemical information regarding the metabolite. Some of the data fields on the right contain hyperlinks (indicated in light blue text). Scroll down to the PubChem (Wheeler et al., 2005) Compound or Substance ID fields and click on one of the hyperlinks on the right. Doing so will open a PubChem Compound or Substance entry for 1-methylhistidine. (You may have to hit the back button to return to the 1-methylhistidine MetaboCard.) Click on the METLIN ID hyperlink to open the METLIN (Smith et al., 2005) page for 1-methlyhistidine. Be sure to hit the Back button to return to the 1-methylhistidine MetaboCard.

In assembling the chemical and biological information contained in the HMDB, more than two dozen textbooks, several thousand journal articles, nearly 30 different electronic databases, and at least 20 in-house or Web-based programs were individually searched, accessed, compared, written, or run over the course of 2 years. The original team of HMDB contributors and annotators included three organic chemists, six NMR spectroscopists, five mass spectroscopists, two separation specialists, three physicians, and fourteen bioinformaticians with dual training in computing science and molecular biology/chemistry (Wishart et al., 2007). Manual updates of the database are continuing, although many annotation fields are now being automatically updated and added using customized text-mining programs.

Table 14.8.1 Summary of the Data Fields or Data Types Found in Each MetaboCard[a]

Metabolite or compound information	Metabolic enzyme information
Common Name	Enzyme Name
Synonyms	Enzyme Synonyms
Chemical IUPAC Name	Enzyme Protein Sequence
Chemical Structure	Enzyme no. of Residues
Chemical Formula	Enzyme Molecular Weight
Chemical Taxonomy	Enzyme pI
Chemical Source	Enzyme Gene Ontology
Molecular Weight	Enzyme General Function
SMILES String	Enzyme Specific Function
KEGG/BioCyc/BiGG/Wikipedia Links	Enzyme Pathways
METLIN/PubChem/ChEBI Links	Enzyme Reactions
CAS Registry no.	Enzyme Pfam Domains
InChI Identifier	Enzyme Signal Sequences
Synthesis Reference	Enzyme Transmembrane Regions
Melting Point	Enzyme Metabolic Importance
Water Solubility	Enzyme EC Link
Physiological Charge	Enzyme GenBank Protein ID
State	Enzyme SwissProt ID
LogP or Hydrophobicity	Enzyme PDB ID
MSDS Link	Enzyme GeneCards ID
MOL/SDF/PDB Text Files	Enzyme Genatlas ID
MOL/PDB Image Files	Enzyme HGNC ID
NMR/MS Spectra	Enzyme 3D Structure
Cellular/Biofluid/Tissue Locations	Enzyme Cellular Location
Normal/Abnormal Concentrations	Enzyme DNA Sequence
Associated Disorders	Enzyme GenBank ID Gene Link
OMIM/Metagene Links	Enzyme Chromosome Location
Pathway Names	Enzyme Locus
KEGG/SimCell Pathway Images	Enzyme SNPs/Mutations
General References	Enzyme General References
Macromolecular Interacting Partners	Enzyme Metabolite References

[a]A more complete listing is provided on the HMDB home page.

5. To view an editable 2-D image of 1-methylhistidine, scroll down to the data field on the MetaboCard page called **MOL File (Image)** and click on the hyperlinked button called **View 2D Structure**. This launches Advanced Chemistry Development's (ACD/Labs) ChemSketch Java applet. After a few seconds, an image of 1-methylhistidine should appear in the applet window (Fig. 14.8.4). If it does not, this likely indicates that your browser lacks the Java Virtual Machine and needs upgrading. To download the necessary Java software, visit *http://www.java.com/getjava/*. The ChemSketch applet used to display this image allows the user to interactively

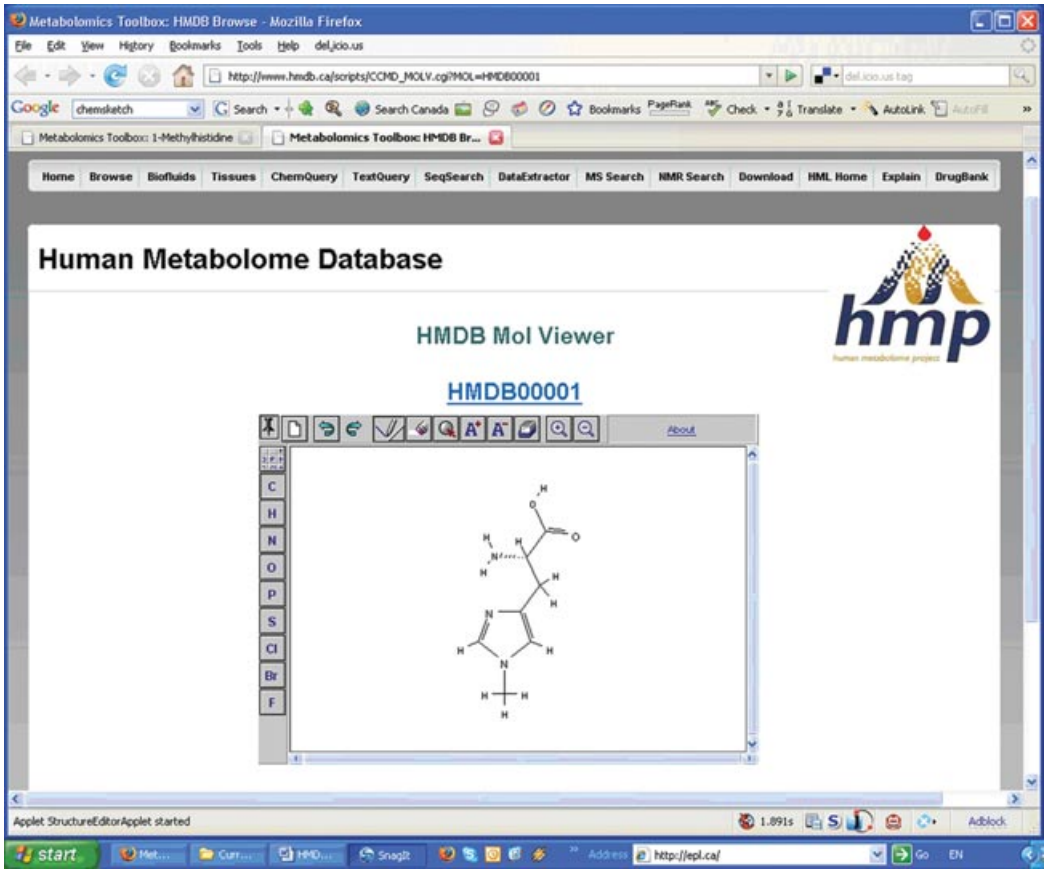


Figure 14.8.4 A 2-D image of the structure of 1-methylhistidine as displayed using the ChemSketch Java applet. The image may be manipulated for different display purposes.

alter, view, rotate, or zoom into the structure and to cut/paste the image (or altered image) into other files.

In addition to structural images of the metabolite, MOL and SDF text files are also available. MOL and SDF files are standard formats used by chemists to exchange and render 2-D chemical structure information. These files can be downloaded by the user and used to display or rerender the structures using higher-end commercial chemistry software packages like ChemDraw, ChemSketch, or ISIS/Draw.

6. A 3-D structure of 1-methylhistidine can also be viewed by clicking on the hyperlinked button **View 3D Structure** contained in the PDB File Calculated (Image) field. This launches the WebMol (Walther, 1997) interactive 3-D viewing applet (Fig. 14.8.5). The Calculated 3-D structure is generated via CORINA (Sadowski and Gasteiger, 1993).

   CORINA is a rule-based structure generation program that has been shown to generate very accurate 3-D structures from 2-D chemical sketches. These calculated structures typically differ from the experimentally determined structures by no more than 0.4 Å. The same WebMol applet that is used to display metabolites in the HMDB can also be used to display protein structures of the metabolic enzymes. WebMol is a fast, flexible viewing tool that allows users to rotate, zoom, color, stereoview, measure, label, and selectively display different parts of a molecule. More information about WebMol and how to use it can be found at http://www.cmpharm.ucsf.edu/~walther/webmol.html.

7. Continue to scroll down the 1-methylhistidine MetaboCard. You should see numerous fields containing detailed nuclear magnetic resonance (NMR) spectral data

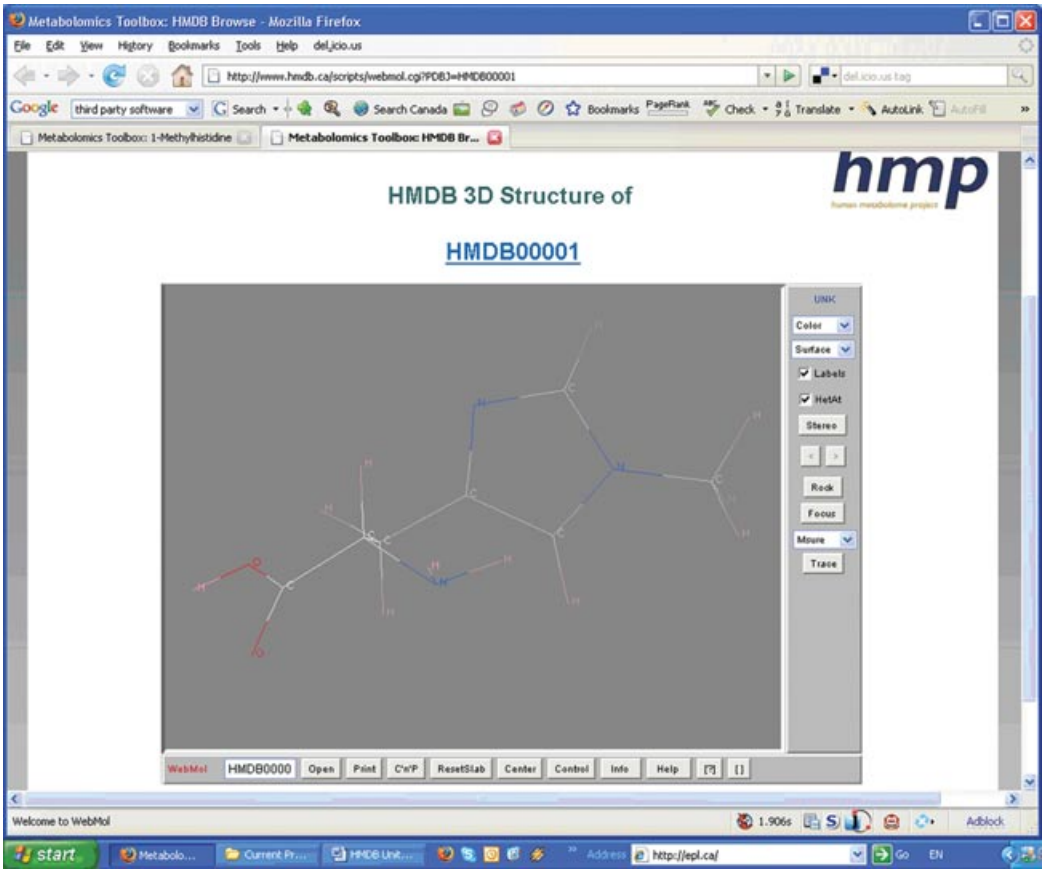


Figure 14.8.5 An image of the 3-D structure of 1-methylhistidine as displayed using the WebMol Java applet. Users may manipulate the image for better viewing or further analysis.

(**Experimental ^{1}H NMR Spectrum**, **Experimental ^{13}C NMR Spectrum**, **Experimental ^{13}C HSQC Spectrum** (Fig. 14.8.6), **Predicted ^{1}H NMR Spectrum**, **Predicted ^{13}C NMR Spectrum**, etc.). Click on these to see what NMR spectral information is available on 1-methylhistidine. The user can also review the experimental conditions by clicking on the **View Experimental Conditions** hyperlink or download the raw FID (free induction decay) file by clicking on the **Download FID** hyperlink for each spectrum. The FID files come in either Varian or Bruker formats depending on the metabolite and type of spectrum (i.e., ^{1}H or ^{13}C). The FID files are the raw binary files used by NMR spectroscopists to render, assign, and manipulate their spectral data. Remember to hit the Back button, once you have viewed the spectral data, to return to the 1-methylhistidine MetaboCard.

The Human Metabolome Database (HMDB) is particularly notable for the amount of NMR spectral information it provides about known human metabolites. Over 1400 experimentally collected ^{1}H and ^{13}C NMR spectra have been assembled for 875 pure compounds (most were collected in water at pH 7.0; 10 mM for ^{1}H, 50 mM for ^{13}C). In addition, there are over 2500 HMDB compounds with predicted ^{1}H and ^{13}C NMR spectra (more than 5000 predicted NMR spectra in total). The predicted spectra are generated using ACD/HNMR and ACD/CNMR software from Advanced Chemistry Development, Inc., with validated MOL files used as the input for each prediction. As will be seen later, these spectra are particularly useful for metabolite identification and verification. While other NMR spectral databases do exist, such as NMRShiftDB (Steinbeck et al., 2003) and the Spectral Database for Organic Compounds (http://riodb01.ibase.aist.go.jp/sdbs/), these are not specific to metabolites, nor are their data typically collected in water near physiological conditions.

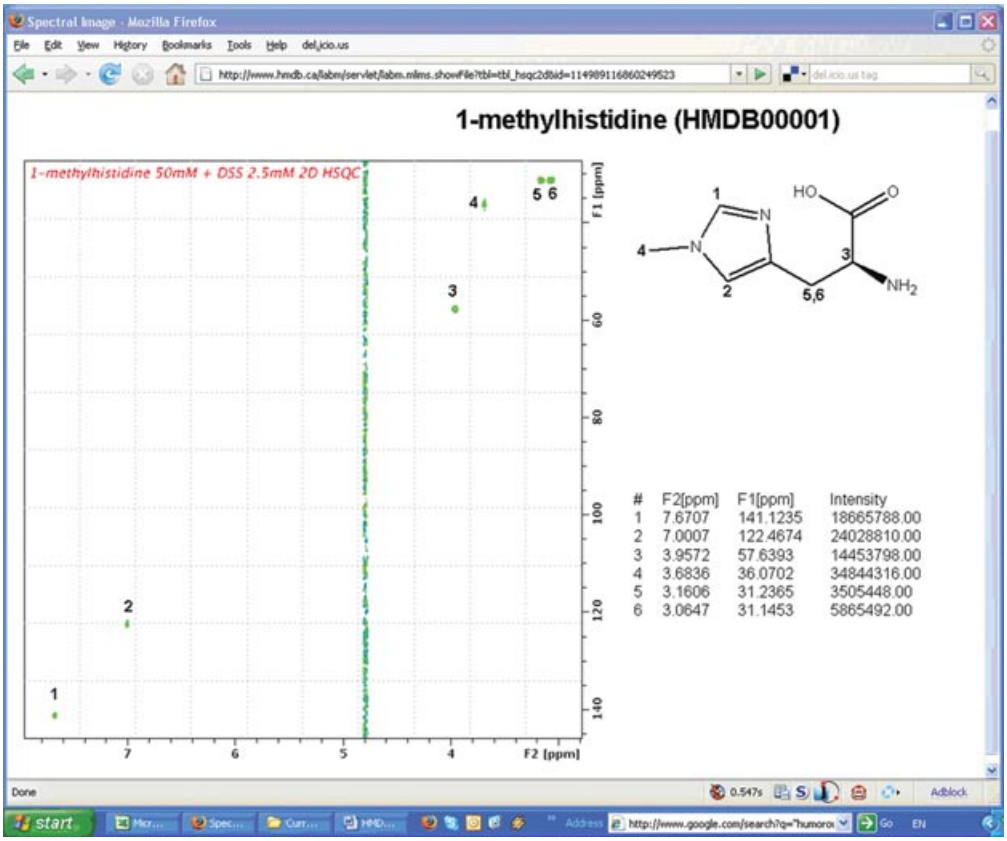


Figure 14.8.6 An image of the 2-D HSQC NMR spectrum and peak list for the metabolite 1-methylhistidine.

8. Below the NMR spectral data is the mass spectrometry data for the 1-methylhistidine MetaboCard. For many of the metabolites in the HMDB, MS/MS (triple quadrupole) spectral data are provided at three collision energies (low, medium, and high). The user can also review the experimental conditions by clicking on the **View Experimental Conditions** hyperlink for each collision energy. For an example of a mass spectrum for 1-methylhistidine, see Figure 14.8.7.

   The HMDB is also notable for the amount of mass spectral data it provides about known human metabolites. Over 1900 experimentally acquired MS/MS (triple quadrupole) spectra have been cataloged for over 660 pure compounds. In total, there are over 2100 MS/MS spectra. In addition to its MS/MS data, the HMDB also includes a GC-MS library with 311 EI spectra and retention times corresponding to 281 metabolites and 30 TMS-derivitization variants. As with the NMR data, these spectra are particularly useful for metabolite identification and verification, as will be shown in Basic Protocol 3.

9. Scroll down further through the 1-methylhistidine MetaboCard. Below the mass spectral, simplified TOCSY spectral, and BMRB spectral data, you should find **Cellular Location**, **Biofluid Location**, and **Tissue Location**. These fields are very useful for anyone wanting to know the location of a particular metabolite within the cell, in the various biofluids (e.g., urine, blood, cerebrospinal fluid, saliva, etc.), or within the different tissue types (e.g., brain, heart, lung, kidney, liver, etc.) throughout the human body. For the cellular location, 1-methylhistidine is normally found in the cytoplasm, while its biofluid locations are in the blood, cerebrospinal fluid (CSF), cellular cytoplasm, saliva, and urine. Its tissue location is limited to muscle and more specifically skeletal muscle.

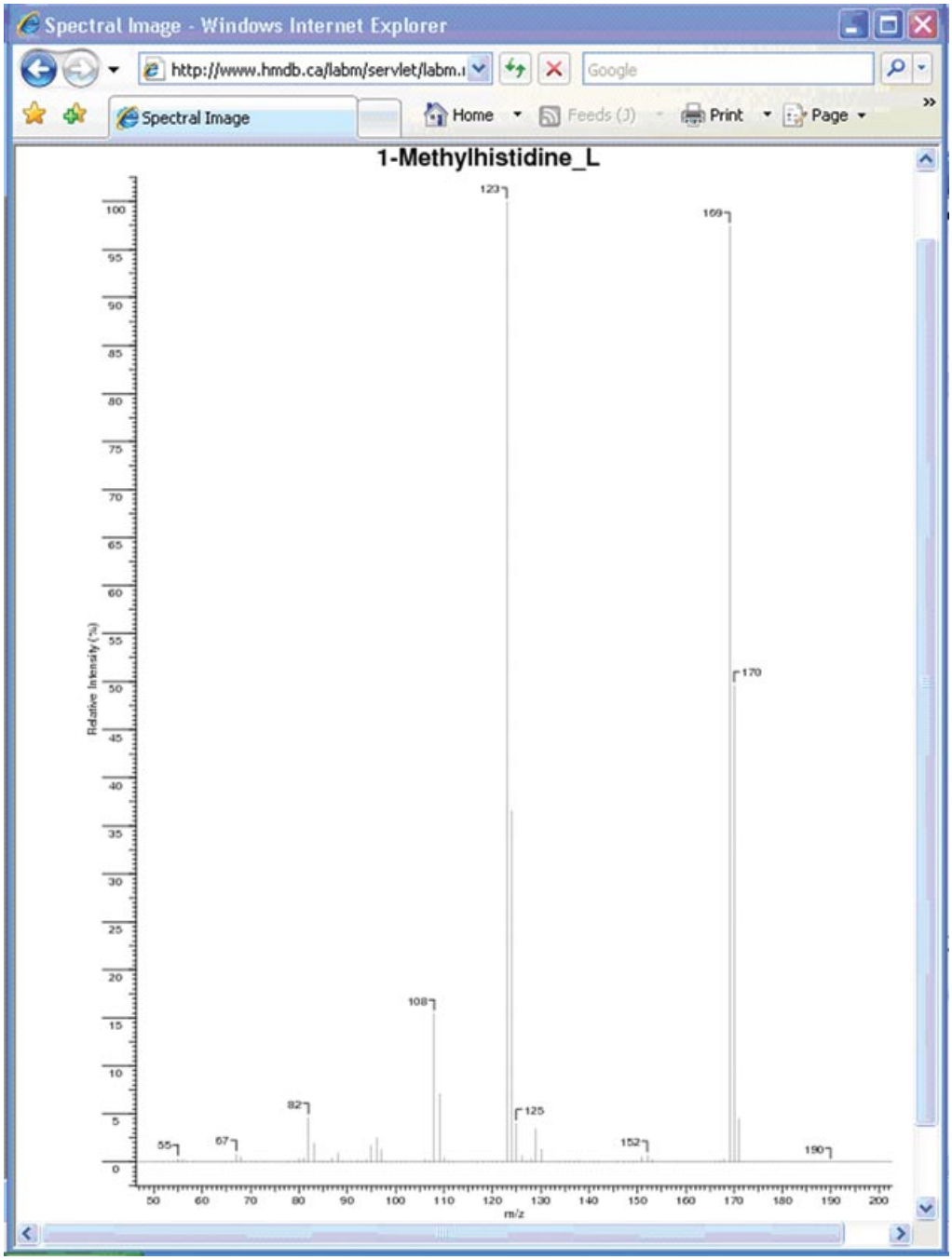
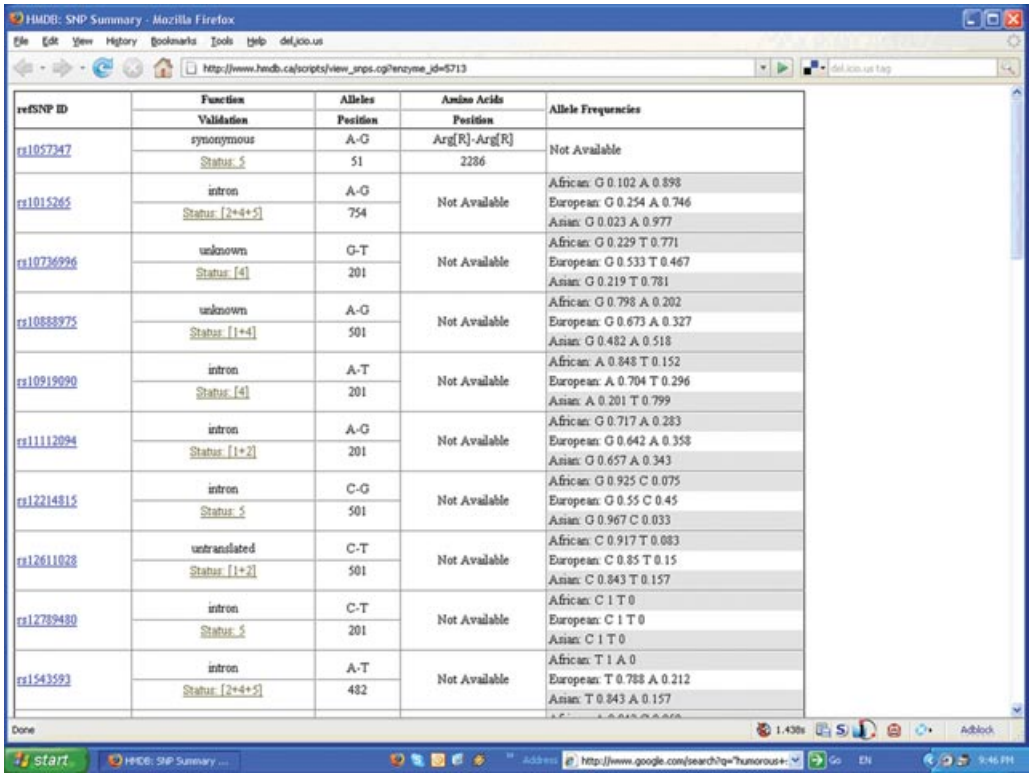


Figure 14.8.7 An image of the MS/MS spectrum at low energy for the metabolite 1-methylhistidine.

10. Scroll down below the location fields to enter into the concentration data section. The right-hand white column is now split into two subcolumns with the sub-fields on the left in bold font (**Biofluid**, **Value**, **Age**, **Sex**, **Condition**, and **References**). For any given MetaboCard, the concentration section can be quite large with many concentration data entries from various literature sources. If you scroll through this section, you will notice that the concentration section has two main fields **Concentration (Normal)** and **Concentration (Abnormal)**. For the normal concentrations, there are sixteen separate literature-derived concentration values with eight from urine, five from blood, one from cerebrospinal fluid (CSF), one from saliva, and one from cellular cytoplasm. For the abnormal concentrations, there are only three



refSNP ID	Function / Validation	Alleles / Position	Amino Acids / Position	Allele Frequencies
rs1057347	synonymous Status: 5	A-G 51	Arg[R]-Arg[R] 2286	Not Available
rs1015265	intron Status: [2+4+5]	A-G 754	Not Available	African: G 0.102 A 0.898 European: G 0.254 A 0.746 Asian: G 0.023 A 0.977
rs10736996	unknown Status: [4]	G-T 201	Not Available	African: G 0.229 T 0.771 European: G 0.533 T 0.467 Asian: G 0.219 T 0.781
rs10888975	unknown Status: [1+4]	A-G 501	Not Available	African: G 0.798 A 0.202 European: G 0.673 A 0.327 Asian: G 0.482 A 0.518
rs10919090	intron Status: [4]	A-T 201	Not Available	African: A 0.848 T 0.152 European: A 0.704 T 0.296 Asian: A 0.201 T 0.799
rs11112094	intron Status: [1+2]	A-G 201	Not Available	African: G 0.717 A 0.283 European: G 0.642 A 0.358 Asian: G 0.657 A 0.343
rs12214815	intron Status: 5	C-G 501	Not Available	African: G 0.925 C 0.075 European: G 0.55 C 0.45 Asian: G 0.967 C 0.033
rs12611028	untranslated Status: [1+2]	C-T 501	Not Available	African: C 0.917 T 0.083 European: C 0.85 T 0.15 Asian: C 0.843 T 0.157
rs12789480	intron Status: 5	C-T 201	Not Available	African: C 1 T 0 European: C 1 T 0 Asian: C 1 T 0
rs1543593	intron Status: [2+4+5]	A-T 482	Not Available	African: T 1 A 0 European: T 0.788 A 0.212 Asian: T 0.843 A 0.157

Figure 14.8.8 Details of the SNP (single nucleotide polymorphism) metabolizing enzyme information contained in the MetaboCard for 1-methylhistidine.

separate entries, two from blood and one from CSF. Another informative field is the **Associated Disorders** field, just below the abnormal concentrations. This section lists different **Conditions** that have been linked to a particular metabolite and provides PubMed and OMIM (Online Mendelian Inheritance in Man; Hamosh et al., 2005) **Reference** hyperlinks.

11. As you scroll down below the **Associated Disorders** field, you will notice a section listing information about known pathways. This information is very useful for anyone wanting to think of the metabolites in terms of their involvement with specific biochemical pathways. The major fields that make up the pathway section include **Pathway Names**, **KEGG Images**, **SimCell Pathway Images**, **SimCell Pathway Graphs**, and **SimCell Pathway SBMLs (View and Download)**. The **Pathway Names** field often provides a succinct two- to four-word descriptor of the pathway that a specific metabolite is involved in. For the visually inclined, the **KEGG Images** field hyperlinks to pathway maps from the KEGG database (Kanehisa et al., 2004). The KEGG pathway maps are generally stored as Graphics Interchange Format (GIF) image files. The next section involves cellular simulation using SimCell (*http://wishart.biology.ualberta.ca/SimCell/*). This program allows users to simulate cellular and biochemical processes using a Dynamic Cellular Automata algorithm (Wishart et al., 2005). In the case of the HMDB, the user can look at simulations of various biochemical pathways involving their metabolite of interest. The **SimCell Pathway Images** field contains hyperlinks to pathway image files that can be used in SimCell to view their metabolite in a simulated pathway. The **SimCell Pathway Graphs** field provides hyperlinks to images of graphs showing the change in the number of molecules of pathway components over time. The **SimCell Pathway SBMLs View** and **Download** fields are more advanced fields that take advantage of

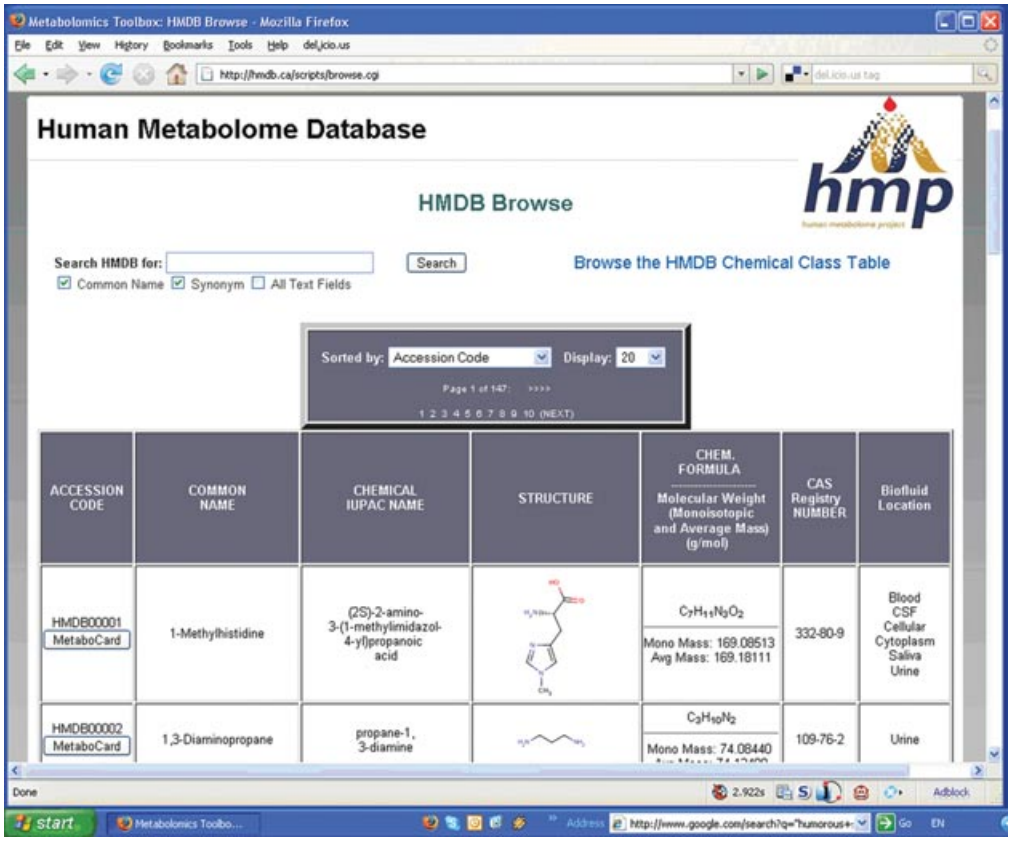


Figure 14.8.9 A screen shot of the HMDB Browser. Note the tabular format and the sorting and display options at the top of the Browser page.

the Systems Biology Markup Language (SBML) to describe models of biochemical reaction networks.

12. Scrolling down further on the 1-methylhistidine MetaboCard, one of the last fields in the first half of the MetaboCard is the **General References** field. This section provides a list of papers that focus on the particular metabolite that is being described, in this case 1-methylhistidine. For each reference, there is a brief citation with author, title, journal, date, volume, number, pages, and, in many cases, a hyperlink to the PubMed entry for the cited reference. At this point, as you scroll down below the **General References** field, you will notice a clearly marked section with the title **Metabolic Enzyme 1**. This section represents the start of the second half of the MetaboCard that is devoted to enzymatic or biochemical data. In other words, this section describes the enzymes that act on the metabolite of interest.

13. As you scroll down the 1-methylhistidine MetaboCard into the **Metabolic Enzyme 1** section, you will notice that this section describes the nomenclature (enzyme name, gene name, synonyms, etc.), gene and protein sequences, other physical properties (e.g., molecular weight and theoretical pI), gene ontology classification, links to external databases, e.g., KEGG pathways (*UNIT 1.12*), Pfam domains (*UNIT 2.5*; Bateman et al., 2004), GenBank (Wheeler et al., 2005), Swiss-Prot (Bairoch et al., 2005), PDB (*UNIT 1.9*), GeneCards (Rebhan et al., 1998), Genatlas (Frézal, 1998), Human Gene Nomenclature Database (HGNC; Wain et al., 2002), etc. As you scroll down past these external links, you will notice a field called **Metabolic Enzyme 1 SNPs**. Click on the hyperlink **View SNPs** to open up a new browser window with a table summarizing many of the known single nucleotide polymorphisms (SNPs) for the

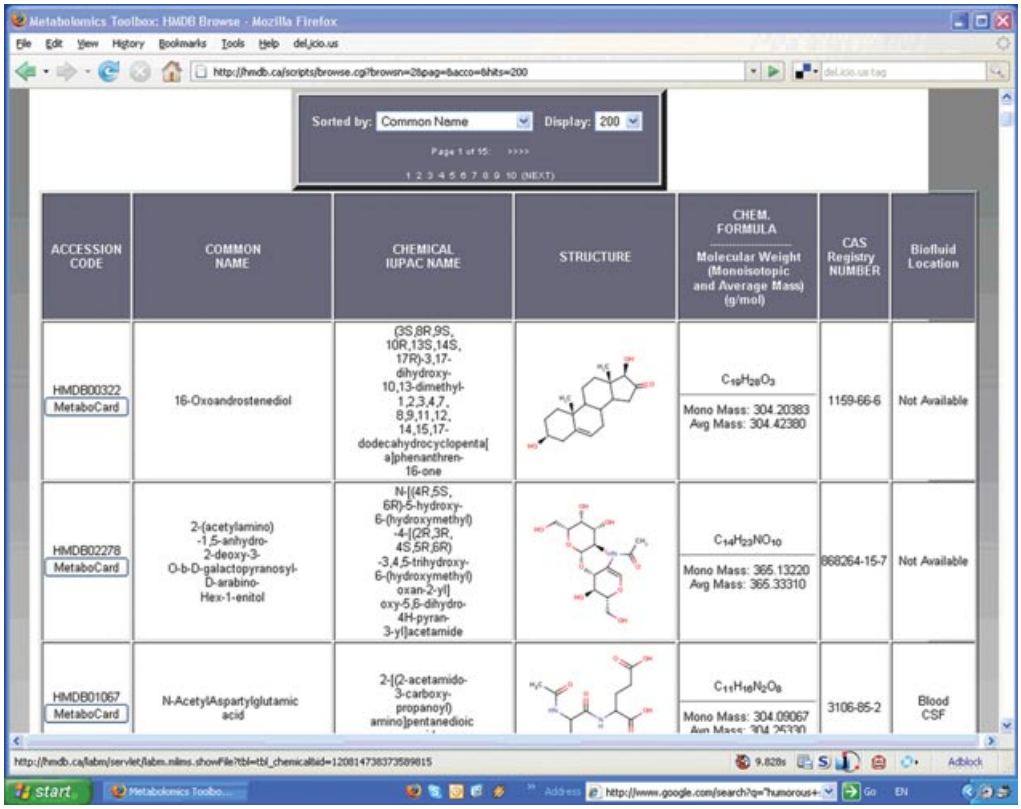


Figure 14.8.10 A screen shot of how the HMDB Browser page should appear when sorting by Common Name and displaying 200 metabolites per page.

metabolizing enzyme of interest, in this case for β-Ala-His dipeptidase (Fig. 14.8.8). This five-column table provides a hyperlink to the refSNP ID, the type of SNP (synonymous or non-synonymous), level of validation, base changes, SNP position, the resulting amino acid changes (if applicable), the amino acid position (if applicable), and the allele frequencies in different populations. The SNP information is particularly important for understanding the origins of certain diseases, the propensity for individuals to get certain diseases, and the type of base changes that are observed in the population as a whole.

Using the Human Metabolome Database Browsers

The following part of the protocol involves learning how to use the different Human Metabolome Database (HMDB) browsing and search tools listed in the HMDB menu bar.

14. Scroll to the top of the MetaboCard for 1-methylhistidine. Click on the Browse hyperlink on the left side of the HMDB menu bar (second from the left) to launch the HMDB Browser (Fig. 14.8.9).

15. Near the top of the Browser page is a dark gray rectangular box that serves as the Browser's sorting and display option interface. On the left side of this box appear the words **Sorted by**. If you click on the right side of this box, on the downward arrow, a pull-down menu appears with different sorting options. Users may sort any given HMDB Browser page by HMDB accession code, common name, chemical IUPAC name, molecular formula, molecular weight, CAS registry number, or biofluid location. Using the **Display** pull-down menu, users may choose to display 20, 50,

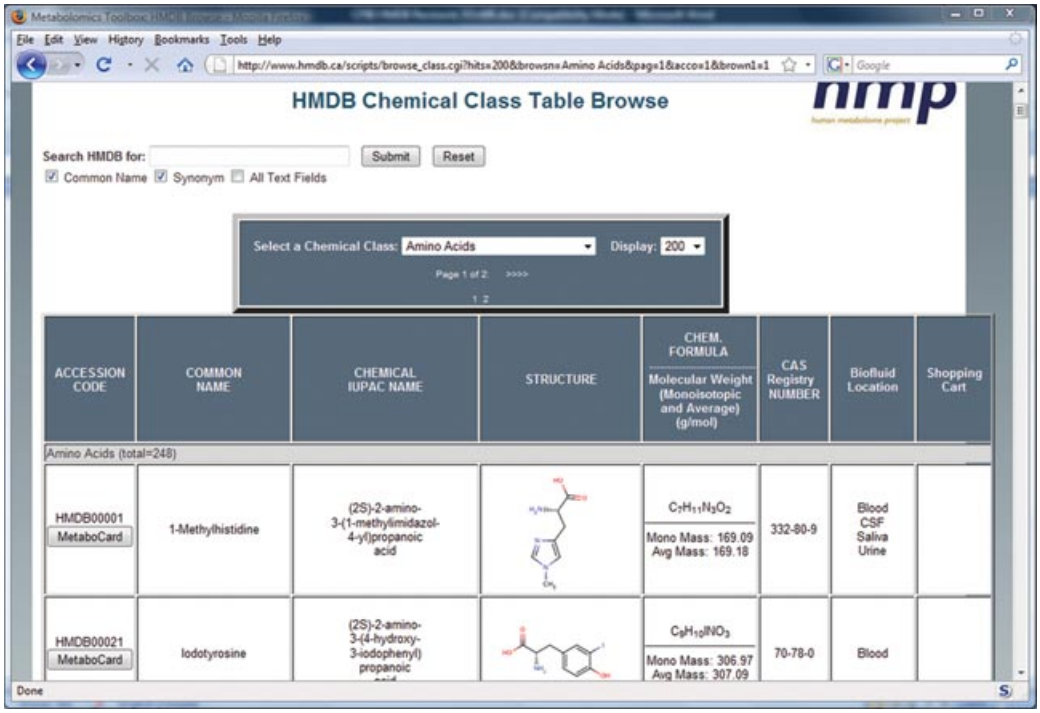
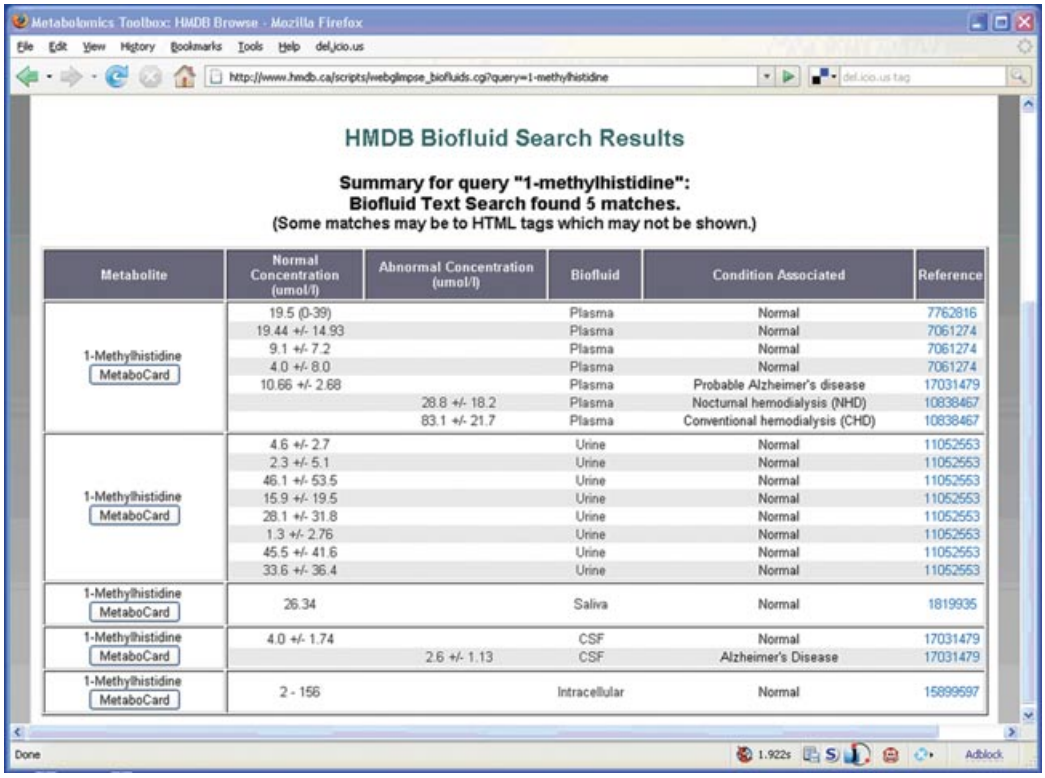


Figure 14.8.11 Here is the result that was obtained with the Chemical Class Browser example (amino acids, display 200 metabolites per page).

100, or 200 metabolites per page. A user may also quickly jump from one page to another using the hyperlinked page numbers or the arrows at the bottom of this box. Sort the table by Common Name and use the Display tab to show 200 metabolites per page. The results should appear as shown in Figure 14.8.10.

16. Return to the top of the Browser page and click on the **Browse the HMDB Chemical Class Table** hyperlink that appears near the top on the right side of the page. This will open the Chemical Class Browser page that allows the user to view metabolites by chemical class. On the Chemical Class Browser page, the user has different display options. A dark gray box appears near the top of the page with the options **Select a Chemical Class** and **Display**. As with the Browser page, the user can also select to display 20, 50, 100, or 200 metabolites per page. The user can also jump to any page using the page numbers and arrows at the bottom of this box. Click on the pull-down menu at the right of the text **Select a Chemical Class** to browse the compounds by chemical class. Select the Amino Acids chemical class and choose to display 200 metabolites per page. The results should fit on two pages since there are a total of 248 metabolites that belong to the amino acid class (Fig. 14.8.11). Note the **Amino Acids (total=248)** that appears on the first page of the Chemical Class Browser table.

There are more than 40 different chemical classes in the HMDB such as amino acids, metal ions or salts, steroids and steroid derivatives, short chain fatty acids, hydroxy acids, alcohols, dicarboxylic acids, etc. Each compound in the HMDB was manually inspected and assigned to a specific chemical class. As yet, there is no definitive taxonomy for metabolites and their corresponding chemical classes. The choice of chemical class assignment was made on the basis of a consensus set of terms commonly used by clinical chemists and metabolomics specialists in the published literature.


Metabolomics Toolbox: HMDB Browse - Mozilla Firefox
File Edit View History Bookmarks Tools Help del.icio.us
http://www.hmdb.ca/scripts/webglimpse_biofluids.cgi?query=1-methylhistidine
HMDB Biofluid Search Results
Summary for query "1-methylhistidine":
Biofluid Text Search found 5 matches.
(Some matches may be to HTML tags which may not be shown.)
Done
Adblock

Metabolite	Normal Concentration (umol/l)	Abnormal Concentration (umol/l)	Biofluid	Condition Associated	Reference
1-Methylhistidine MetaboCard	19.5 (0-39)		Plasma	Normal	7762816
	19.44 +/- 14.93		Plasma	Normal	7061274
	9.1 +/- 7.2		Plasma	Normal	7061274
	4.0 +/- 8.0		Plasma	Normal	7061274
	10.66 +/- 2.68		Plasma	Probable Alzheimer's disease	17031479
		28.8 +/- 18.2	Plasma	Nocturnal hemodialysis (NHD)	10838467
		83.1 +/- 21.7	Plasma	Conventional hemodialysis (CHD)	10838467
1-Methylhistidine MetaboCard	4.6 +/- 2.7		Urine	Normal	11052553
	2.3 +/- 5.1		Urine	Normal	11052553
	46.1 +/- 53.5		Urine	Normal	11052553
	15.9 +/- 19.5		Urine	Normal	11052553
	28.1 +/- 31.8		Urine	Normal	11052553
	1.3 +/- 2.76		Urine	Normal	11052553
	45.5 +/- 41.6		Urine	Normal	11052553
	33.6 +/- 36.4		Urine	Normal	11052553
1-Methylhistidine MetaboCard	26.34		Saliva	Normal	1819935
1-Methylhistidine MetaboCard	4.0 +/- 1.74		CSF	Normal	17031479
		2.6 +/- 1.13	CSF	Alzheimer's Disease	17031479
1-Methylhistidine MetaboCard	2 - 156		Intracellular	Normal	15899597

Figure 14.8.12 1-methylhistidine appears in five different biofluids. This result was obtained using a search for this particular metabolite in different biofluids from the Biofluid Browser page.

17. Click on the **Biofluids** hyperlink (third from the left) on the HMDB menu. This will open the HMDB Biofluid Browser. As with the other two HMDB Browsers, the Biofluid Browser includes many options. With this Browser, the user can search for specific metabolites by biofluid location or by concentration range. In terms of display options, the user may display metabolites by biofluid type and sort the results by common name (ascending or descending order), normal concentration (ascending or descending order), or associated condition (ascending or descending order). The different biofluid locations include: plasma/serum, urine, saliva, gallbladder, cerebrospinal fluid (CSF), intracellular, and breast milk. In the text box that appears to the right of the text **Search Biofluid for**, type `1-methylhistidine` and hit the Search button to display a table listing all of the biofluid locations for this particular metabolite. The results should show that 1-methylhistidine appears in five different biofluids: plasma, urine, saliva, CSF, and intracellular (Fig. 14.8.12).

18. Return to the Biofluid Browser home page (*http://hmdb.ca/scripts/Biofluid_browse.cgi*) by clicking on the Biofluids hyperlink on the HMDB menu. Below the search fields appears a gray box with **Select Biofluid Type** and **Sorted by**. Click on the downward arrow to the right of **Select Biofluid Type** to select "Gallbladder" and click on the downward arrow to the right of **Sorted by** to select "Associated Condition (ascending)."

    This exercise should reveal a select list of metabolites from gallbladder bile with associated disorders: cholesterol, bilirubin, lecithin, L-lactic acid, and palmitic acid (Fig. 14.8.13).

19. The HMDB Tissue Browser allows users to browse the database by tissue. Scroll back up to the top of the Biofluid Browser home page and click on the **Tissues**

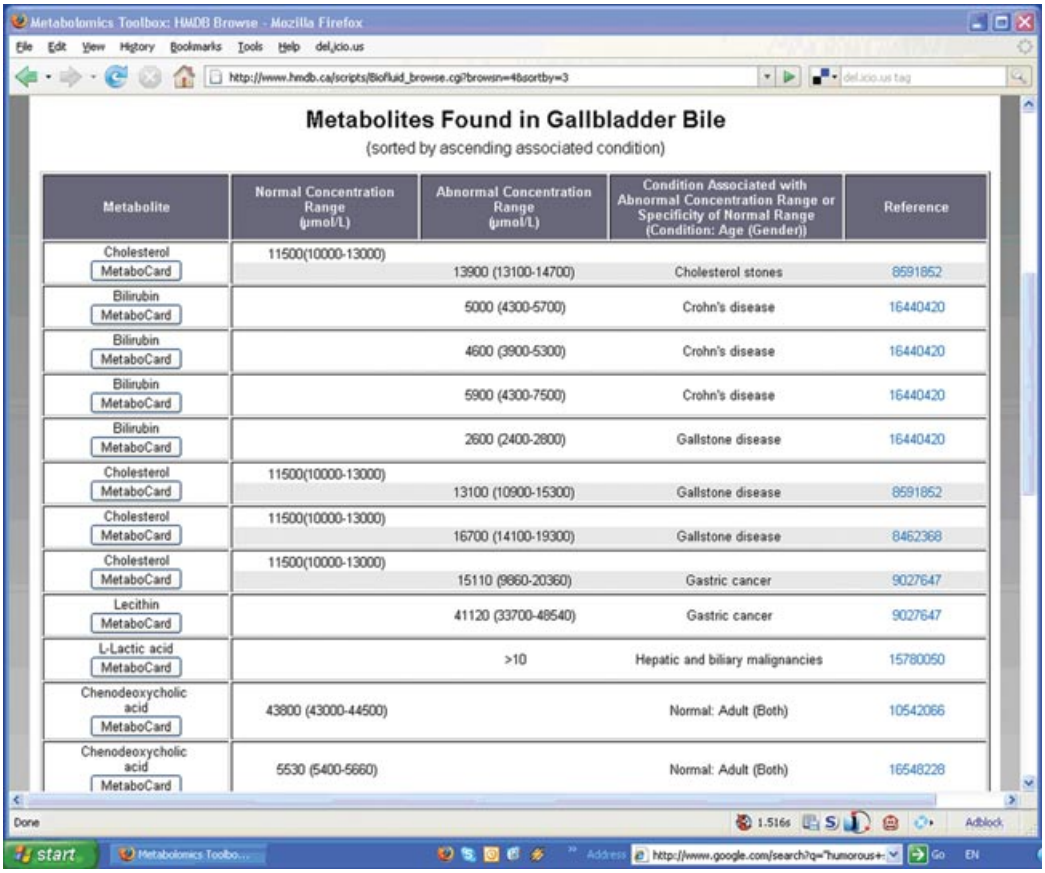

Metabolomics Toolbox: HMDB Browse - Mozilla Firefox
File Edit View History Bookmarks Tools Help del.icio.us
http://www.hmdb.ca/scripts/Biofluid_browse.cgi?browsn=48sortby=3
Metabolites Found in Gallbladder Bile
(sorted by ascending associated condition)
Metabolite
Normal Concentration Range (μmol/L)
Abnormal Concentration Range (μmol/L)
Condition Associated with Abnormal Concentration Range or Specificity of Normal Range (Condition: Age (Gender))
Reference
Cholesterol MetaboCard 11500(10000-13000) 13900 (13100-14700) Cholesterol stones 8591852
Bilirubin MetaboCard 5000 (4300-5700) Crohn's disease 16440420
Bilirubin MetaboCard 4600 (3900-5300) Crohn's disease 16440420
Bilirubin MetaboCard 5900 (4300-7500) Crohn's disease 16440420
Bilirubin MetaboCard 2600 (2400-2800) Gallstone disease 16440420
Cholesterol MetaboCard 11500(10000-13000) 13100 (10900-15300) Gallstone disease 8591852
Cholesterol MetaboCard 11500(10000-13000) 16700 (14100-19300) Gallstone disease 8462368
Cholesterol MetaboCard 11500(10000-13000) 15110 (9860-20360) Gastric cancer 9027647
Lecithin MetaboCard 41120 (33700-48540) Gastric cancer 9027647
L-Lactic acid MetaboCard >10 Hepatic and biliary malignancies 15780050
Chenodeoxycholic acid MetaboCard 43800 (43000-44500) Normal: Adult (Both) 10542066
Chenodeoxycholic acid MetaboCard 5530 (5400-5660) Normal: Adult (Both) 16548228
Done
start

Figure 14.8.13 Restricting the search to metabolites from gallbladder bile with associated disorders limits the number of metabolites to a total of five.

hyperlink just to the right of **Biofluids** on the HMDB menu bar. This will open up the HMDB Tissue Browser page. By default, all of the metabolites in the HMDB are displayed in a table with three columns: HMDB ID, Name, and Tissue. Note that above each column header there are empty boxes. Users can input their query data in these boxes and press the Search button to launch their search. Clicking on one of the column headers sorts the column by that field (one click sorts by ascending order and two clicks by descending order). For example, if a user clicks on the HMDB ID, all of the metabolites are sorted in ascending order, from HMDB00001 to HMDB05176. If one wanted to look at all metabolites with the word "thyroid" in the tissue field, then `thyroid` should be typed in the third column header query field, and the Search button should be clicked. In this case, the Tissue Browser returns a total of 53 matches (Fig. 14.8.14). The user can navigate the hit results using the **First**, **Previous**, **Next**, and **Last** arrows near the top of the page. The user can also select to display 50, 100, or 500 metabolites per page. The data can also be exported to Microsoft Excel by clicking on the **Export XLS** hyperlink.

20. Scroll back up to the top of the Tissue Browser home page and click on the **TextQuery** hyperlink located just to the right of the **ChemQuery** hyperlink on the HMDB Menu. This will open the HMDB Text Search page. This page provides many user options including text or numerical searches by common name, synonym, or all text fields. The user can also select case sensitivity, partial matches, and up to two misspellings. In addition, the user can choose to display the top 10, 20, 50, 100, 200, 500, or 1000 hits. For instance, users interested in finding out which metabolites are associated

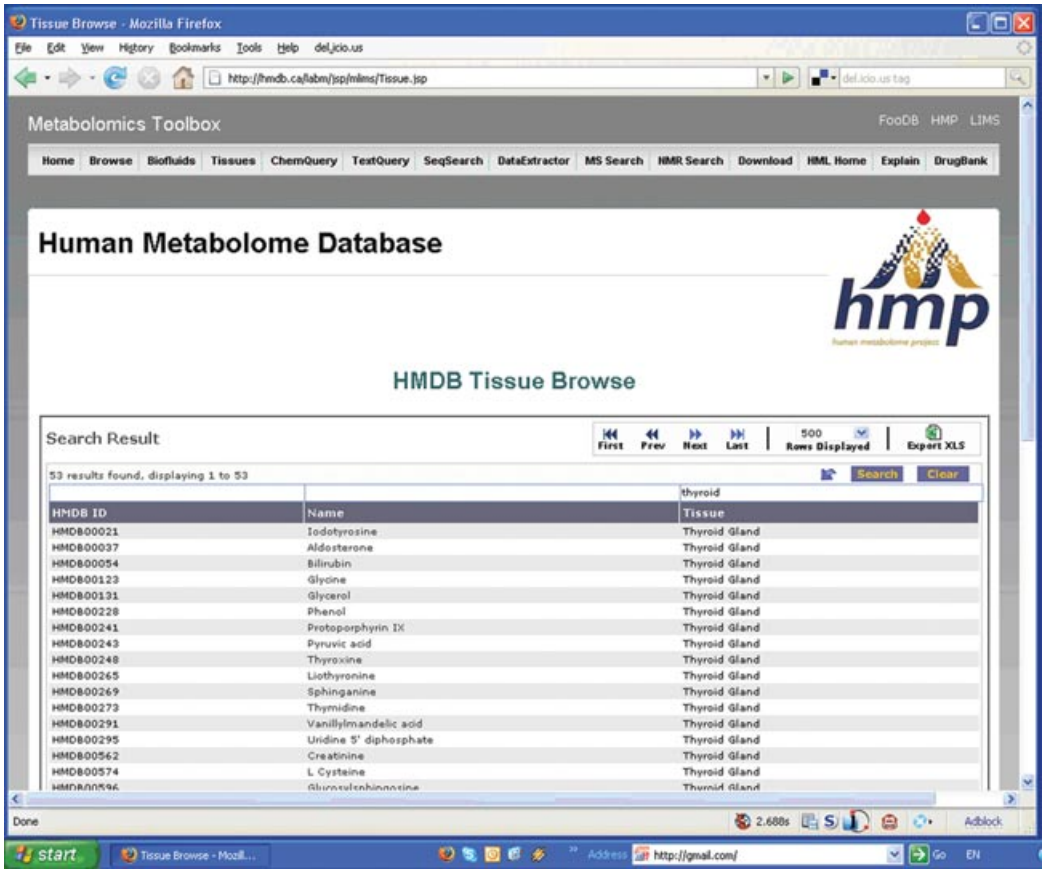

Tissue Browse - Mozilla Firefox
Metabolomics Toolbox
Home Browse Biofluids Tissues ChemQuery TextQuery SeqSearch DataExtractor MS Search NMR Search Download HML Home Explain DrugBank
Human Metabolome Database
HMDB Tissue Browse
Search Result
53 results found, displaying 1 to 53
HMDB ID Name Tissue
HMDB00021 Iodotyrosine Thyroid Gland
HMDB00037 Aldosterone Thyroid Gland
HMDB00054 Bilirubin Thyroid Gland
HMDB00123 Glycine Thyroid Gland
HMDB00131 Glycerol Thyroid Gland
HMDB00228 Phenol Thyroid Gland
HMDB00241 Protoporphyrin IX Thyroid Gland
HMDB00243 Pyruvic acid Thyroid Gland
HMDB00248 Thyroxine Thyroid Gland
HMDB00265 Liothyronine Thyroid Gland
HMDB00269 Sphinganine Thyroid Gland
HMDB00273 Thymidine Thyroid Gland
HMDB00291 Vanillylmandelic acid Thyroid Gland
HMDB00295 Uridine 5' diphosphate Thyroid Gland
HMDB00562 Creatinine Thyroid Gland
HMDB00574 L Cysteine Thyroid Gland

Figure 14.8.14 In this example of the HMDB Tissue Browser, only metabolites from the thyroid are displayed.

with obesity would type `obesity` in the **Search for:** text box, making sure that the **Common Name**, **Synonym**, and **All Text Fields** boxes are checked. Note that **Partial Match** can be checked or unchecked, with no change in the results. After hitting the Submit button, a new browser window should open with a table of 58 metabolites that match this text query (Fig. 14.8.15). If one wanted to limit a search, the search could be repeated as above, but this time typing `obesity AND stroke`. This would generate a list of metabolites that contain "obesity" and "stroke" somewhere in the MetaboCard. This time, the TextQuery Browser returns only two matches, cholesterol and taurine.

21. At this point, the user will learn how to use the HMDB Data Extractor. This tool allows users with no database experience to build powerful Structured Query Language (SQL)-based queries without having to know SQL. SQL is the computer language used to provide an interface to relational databases (see *UNIT 9.2*). To begin, we need to scroll back up to the HMDB menu near the top of most HMDB pages. Click on the **Data Extractor** hyperlink in the middle of the HMDB menu. This will open a new window with two frames; the frame on the left allows users to select various fields that they would like to use to search the database. For example, if a user wanted to build a query form that allowed them to search for all metabolites with a molecular weight that is between 145 and 155 Da, a melting point between 0° and 100°C, a biofluid location that is blood, and a tissue location that is erythrocyte (red blood cell), they would select the following fields: "molecular weight," "melting point," "biofluid location," and "tissue location," *holding down the control (Ctrl) key to*

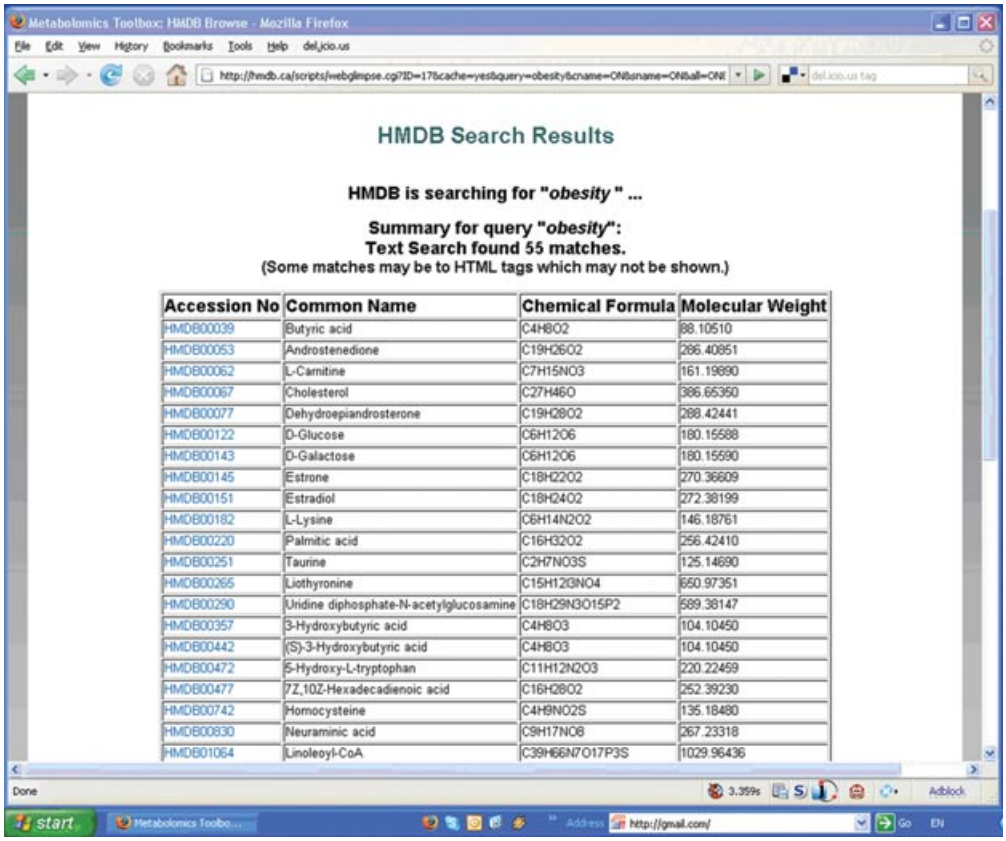

Metabolomics Toolbox: HMDB Browse - Mozilla Firefox
HMDB Search Results
HMDB is searching for "obesity" ...
Summary for query "obesity":
Text Search found 55 matches.
(Some matches may be to HTML tags which may not be shown.)
Accession No | Common Name | Chemical Formula | Molecular Weight
HMDB00039 | Butyric acid | C4H8O2 | 88.10510
HMDB00053 | Androstenedione | C19H26O2 | 286.40851
HMDB00062 | L-Carnitine | C7H15NO3 | 161.19890
HMDB00067 | Cholesterol | C27H46O | 386.65350
HMDB00077 | Dehydroepiandrosterone | C19H28O2 | 288.42441
HMDB00122 | D-Glucose | C6H12O6 | 180.15588
HMDB00143 | D-Galactose | C6H12O6 | 180.15590
HMDB00145 | Estrone | C18H22O2 | 270.36609
HMDB00151 | Estradiol | C18H24O2 | 272.38199
HMDB00182 | L-Lysine | C6H14N2O2 | 146.18761
HMDB00220 | Palmitic acid | C16H32O2 | 256.42410
HMDB00251 | Taurine | C2H7NO3S | 125.14690
HMDB00265 | Liothyronine | C15H12I3NO4 | 650.97351
HMDB00290 | Uridine diphosphate-N-acetylglucosamine | C18H29N3O15P2 | 589.38147
HMDB00357 | 3-Hydroxybutyric acid | C4H8O3 | 104.10450
HMDB00442 | (S)-3-Hydroxybutyric acid | C4H8O3 | 104.10450
HMDB00472 | 5-Hydroxy-L-tryptophan | C11H12N2O3 | 220.22459
HMDB00477 | 7Z,10Z-Hexadecadienoic acid | C16H28O2 | 252.39230
HMDB00742 | Homocysteine | C4H9NO2S | 135.18480
HMDB00830 | Neuraminic acid | C9H17NO8 | 267.23318
HMDB01064 | Linoleoyl-CoA | C39H66N7O17P3S | 1029.96436

Figure 14.8.15 In this example of the HMDB TextQuery Tool, only metabolites that contain text with the word `obesity` are displayed.

select noncontiguous fields. Once the fields of interest are selected, the user must click on the Go button to build the query form (Fig. 14.8.16). The form will appear in the right-side frame. With this form, a user may enter values in the boxes that appear on the right. In the molecular weight boxes, enter `145` and `150`, while in the melting point fields, enter `0` and `100`. For the biofluid location and tissue location fields, enter `blood` and `erythrocyte`, respectively. Hit the Submit button to launch the query. A new window opens in the right frame with the results. There is only one hit from the database that matches this complex query: spermidine. In a similar way, users can build complex queries to search the metabolic enzymes and macromolecular interacting partners that make up the other important half of the database.

Using the download page

22. Return to the HMDB menu at the top of the right frame of the **Data Extractor** page and click on the **Download** hyperlink. The Download page window as shown in Figure 14.8.17 should appear. This page provides access to much of the HMDB's downloadable content including protein and DNA sequences in FASTA format (redundant and nonredundant sets), MetaboCard flat files and MetaboCard hyperlinks, structure files in various formats (SDF, MOL, PDB, canonical SMILES strings), and spectral files (MS and NMR). Toward the bottom of this page, up-to-date statistics about the HMDB downloadable content is provided, as shown in Figure 14.8.18.

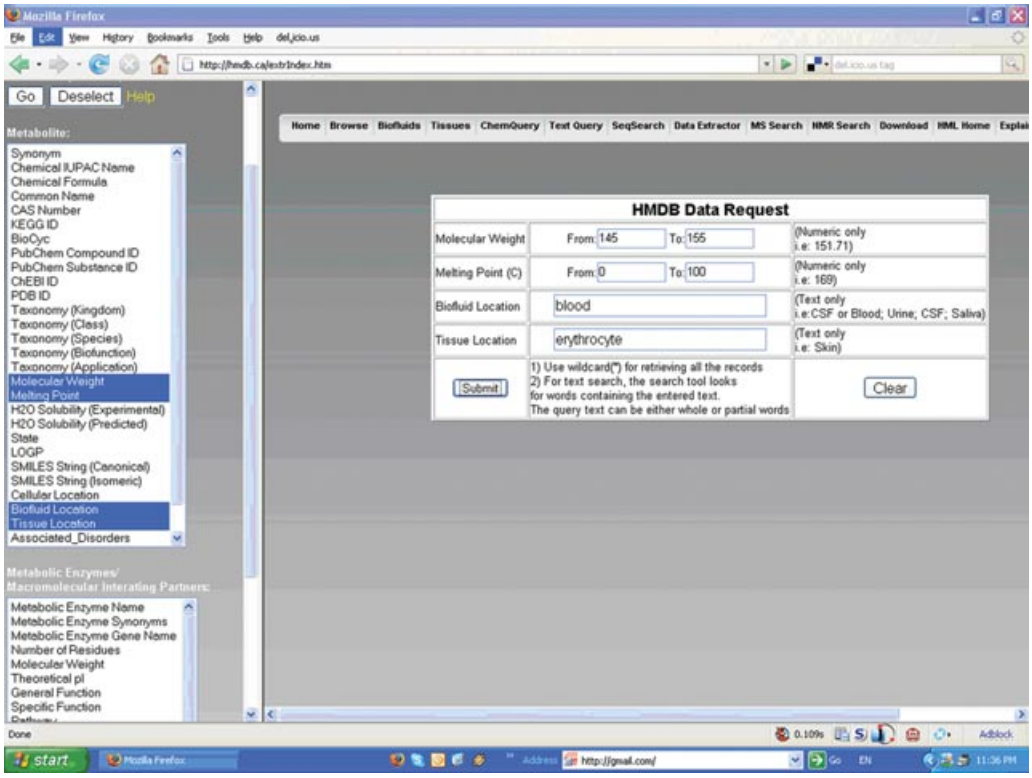


Figure 14.8.16 The HMDB Data Extractor can be used to build complex queries as shown here.

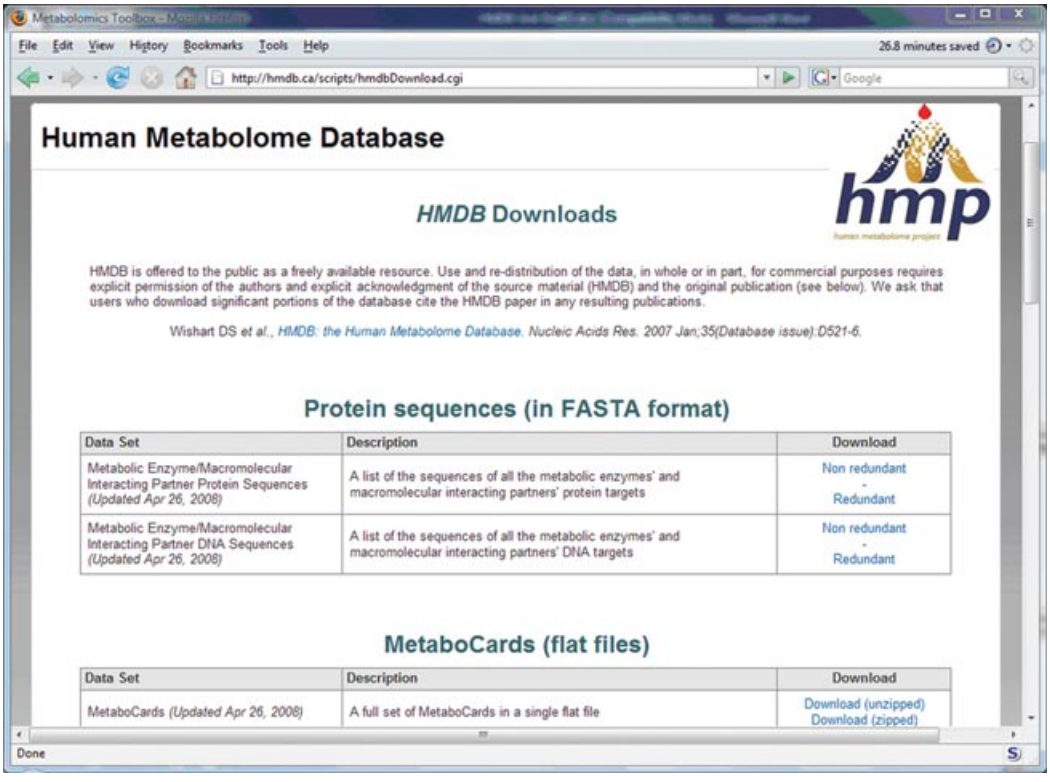

Metabolomics Toolbox
File
Edit
View
History
Bookmarks
Tools
Help
http://hmdb.ca/scripts/hmdbDownload.cgi
Human Metabolome Database
HMDB Downloads
HMDB is offered to the public as a freely available resource. Use and re-distribution of the data, in whole or in part, for commercial purposes requires explicit permission of the authors and explicit acknowledgment of the source material (HMDB) and the original publication (see below). We ask that users who download significant portions of the database cite the HMDB paper in any resulting publications.
Wishart DS et al., HMDB: the Human Metabolome Database. Nucleic Acids Res. 2007 Jan;35(Database issue):D521-6.
Protein sequences (in FASTA format)
Data Set
Description
Download
Metabolic Enzyme/Macromolecular Interacting Partner Protein Sequences (Updated Apr 26, 2008)
A list of the sequences of all the metabolic enzymes' and macromolecular interacting partners' protein targets
Non redundant
Redundant
Metabolic Enzyme/Macromolecular Interacting Partner DNA Sequences (Updated Apr 26, 2008)
A list of the sequences of all the metabolic enzymes' and macromolecular interacting partners' DNA targets
Non redundant
Redundant
MetaboCards (flat files)
Data Set
Description
Download
MetaboCards (Updated Apr 26, 2008)
A full set of MetaboCards in a single flat file
Download (unzipped)
Download (zipped)
Done

Figure 14.8.17 The HMDB Download page provides access to many large, downloadable text files containing much of the HMDB's content.

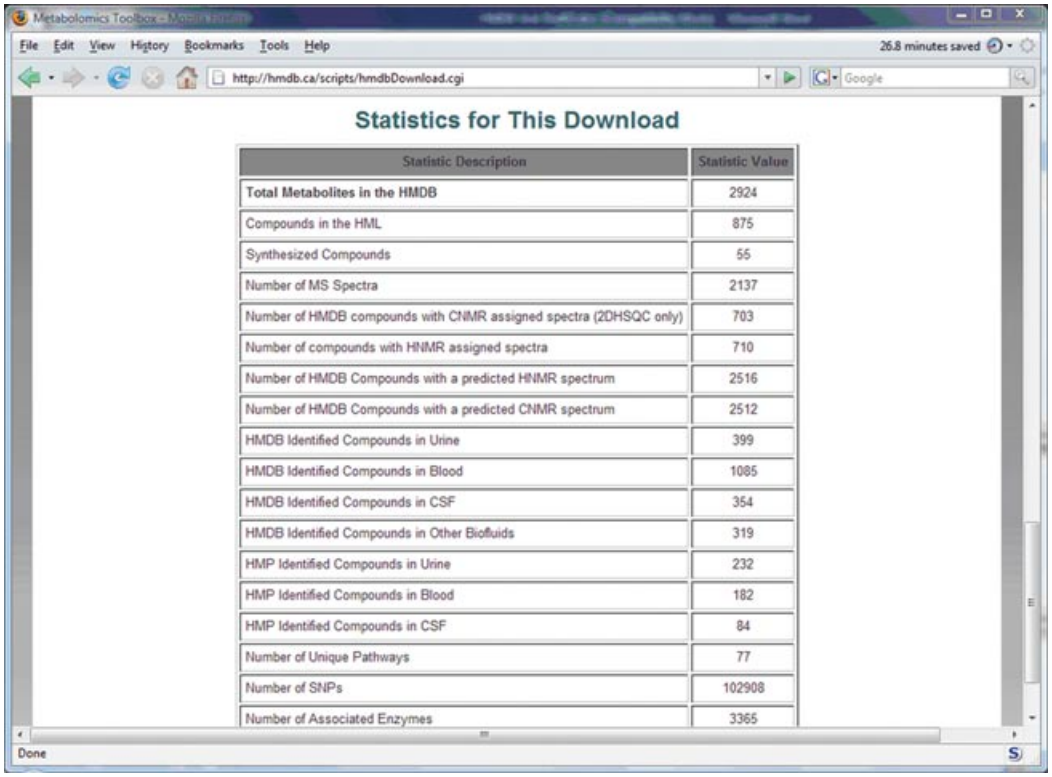

Metabolomics Toolbox - Mozilla Firefox
File Edit View History Bookmarks Tools Help
26.8 minutes saved
http://hmdb.ca/scripts/hmdbDownload.cgi
Google
Statistics for This Download
Statistic Description
Statistic Value
Total Metabolites in the HMDB 2924
Compounds in the HML 875
Synthesized Compounds 55
Number of MS Spectra 2137
Number of HMDB compounds with CNMR assigned spectra (2DHSQC only) 703
Number of compounds with HNMR assigned spectra 710
Number of HMDB Compounds with a predicted HNMR spectrum 2516
Number of HMDB Compounds with a predicted CNMR spectrum 2512
HMDB Identified Compounds in Urine 399
HMDB Identified Compounds in Blood 1085
HMDB Identified Compounds in CSF 354
HMDB Identified Compounds in Other Biofluids 319
HMP Identified Compounds in Urine 232
HMP Identified Compounds in Blood 182
HMP Identified Compounds in CSF 84
Number of Unique Pathways 77
Number of SNPs 102908
Number of Associated Enzymes 3365
Done

Figure 14.8.18 Near the bottom of the HMDB Download page, up-to-date statistics about the HMDB downloadable content is provided.

BASIC PROTOCOL 2

CHEMICAL STRUCTURE SIMILARITY SEARCHING

In cheminformatics, metabolite searches based on chemical structure similarity are analogous to sequence searches based on sequence similarity or structural searches based on similarity to 3-D structures such as those found in the Protein Data Bank (PDB; *UNIT 4.9*). Chemical structure similarity searches may be especially useful for organic chemists and analytical chemists wanting to determine whether a newly synthesized or newly identified compound shows similarity to a known metabolite. Chemical structure similarity searches may also prove to be very useful when looking at compounds with the same parent compound or from the same chemical class. In some cases, chemical structure similarity searching can be more powerful than text-based searching. Often, the naming conventions for various metabolites are inconsistent, and there are sometimes spelling errors that can make text-based searching challenging. In many cases, searching for a precise chemical structure match or similar chemical structure match can lead to very informative results, while some text-based searches may yield no adequate results. This protocol describes some of the features of the HMDB's chemical structure-based search methods.

Necessary Resources

Hardware

Computer with Internet access

Software

An up-to-date Web browser, such as Internet Explorer (*http://www.microsoft.com/ie/*), Firefox (*http://www.mozilla.com/*), Netscape (*http://browser.netscape.com/*), Opera (*http://www.opera.com/*), or Safari (*http://www.apple.com/safari/*). The Web browser must be capable of handling Java applets (i.e., equipped with a recent version of the Java interpreter).

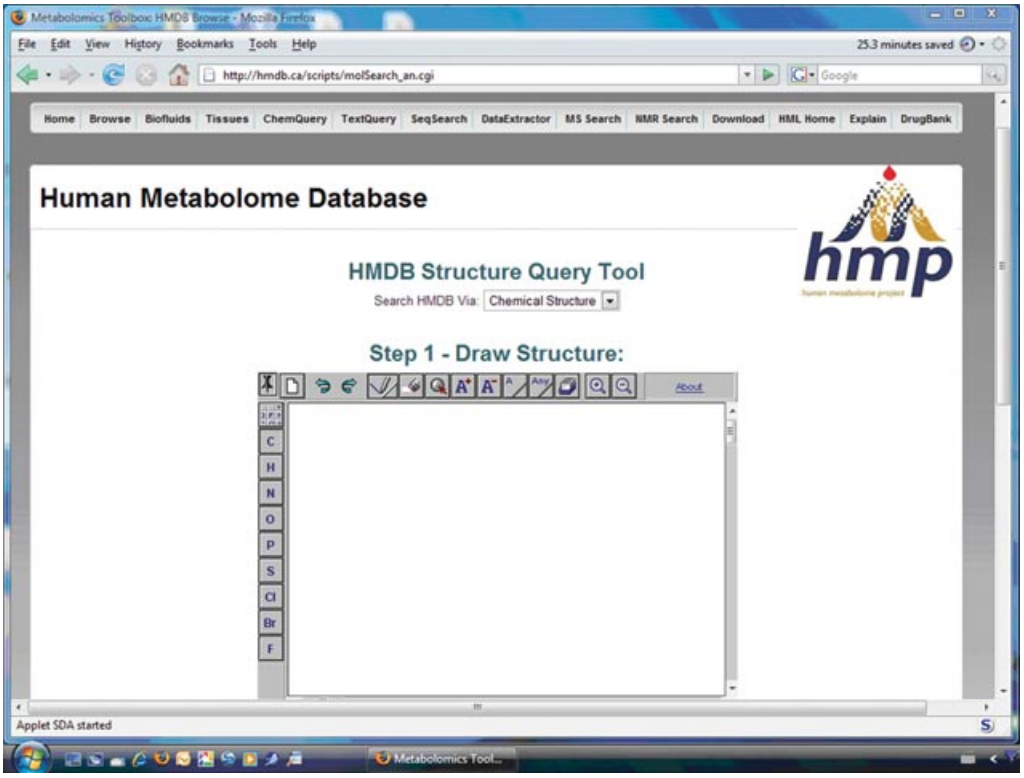


Figure 14.8.19 In this particular view of the HMDB ChemQuery home page, the chemical structure drawing applet window is shown.

Files

None

Using ChemQuery

1. Go to the Human Metabolome Database Web site at *http://hmdb.ca/*.

   The HMDB home page should appear along with the light gray menu bar located near the top of the page with fourteen clickable links: Home, Browse, Biofluids, Tissues, ChemQuery, TextQuery, SeqSearch, DataExtractor, MS/MS Search, MS Search, GC/MS Search, NMR Search, Download, and Explain.

2. Click on the **ChemQuery** hyperlink on the HMDB menu (fifth hyperlink from the left). A window should appear with a pull-down menu and the ACD/Structure Drawing Java applet (Fig. 14.8.19). The pull-down menu (**Search HMDB Via**) allows users to select the type of chemical structure search. Users can search by Chemical Structure, Molecular Weight, Chemical Formula, or SMILES String.

   The ChemQuery tool provides a wide variety of chemical structure query options. Using the "Search HMDB Via:" pull-down menu, users can either draw chemical structures using the ACD/Structure Drawing applet, enter a range of molecular weights (using text boxes), type the chemical formula with flexibility in being able to search for compounds matching a range of chemical formulas, or enter a SMILES string (Weininger, 1988). Mass spectroscopists are generally interested in searching for compounds by chemical formula or molecular weight ranges, since the data generated by mass spectrometers (MS) are typically mass ranges and approximate chemical formulas. SMILES string and chemical structure searching are typically more useful for organic and natural product chemists as well as biochemists. The SMILES (Simplified Molecular Input Line Entry Specification) string unambiguously describes the structure of chemical compounds using short ASCII strings. Most molecule editors can be used to import SMILES strings and convert them into 2-D drawings or 3-D molecular models.

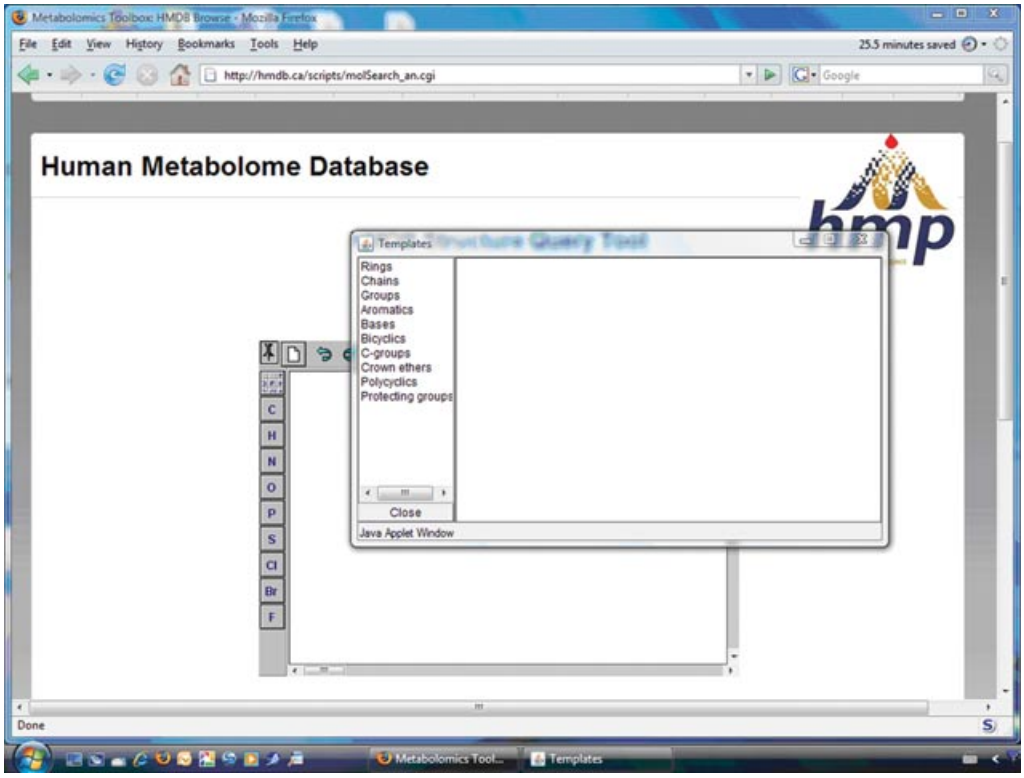


Figure 14.8.20 A variety of chemical structure templates are available to reduce some of the manual drawing requirements.

Drawing a chemical structure using ChemQuery

This particular part of the protocol will use the ChemQuery tool to look for small molecules that are similar in chemical structure to the neurotransmitter dopamine. The user should leave the ChemQuery pull-down menu on the default setting (**Chemical Structure**).

3. To begin drawing the chemical structure for dopamine, look for the row of buttons above the structure drawing applet window and click on what looks like a stack of index cards (third button from the right). This will open up a template of available chemical structures. A separate window should appear with a list of different structure template names (**Rings**, **Chains**, **Groups**, **Aromatics**, etc.) on the left side (see Fig. 14.8.20).

   The ACD (Advanced Chemistry Development)/Structure Drawing applet is relatively easy to use. On the left side of the applet is a column of buttons for adding the commonly used chemical elements (carbon, hydrogen, nitrogen, oxygen, phosphorus, sulfur, chlorine, bromine, and fluorine). Above the C (carbon) is a button that gives access to the remaining chemicals from the periodic table of elements. At the top of the applet are a number of buttons for drawing, erasing, moving, zooming, undoing, redoing, and clearing different drawing elements or drawings. When the user mouses over each of these buttons, a brief one or two word description of the button appears in the upper right corner of the applet. Clicking on the About hyperlink in the upper right corner opens a new window with more details about the ACD/Structure Drawing applet. The easiest way to quickly draw structures is to take advantage of the chemical structure templates collection (the button that looks like a stack of index cards).

4. Select the **Aromatics** template gallery by clicking on the word Aromatics, listed fourth on the template list. In the right pane of the template window, a collection

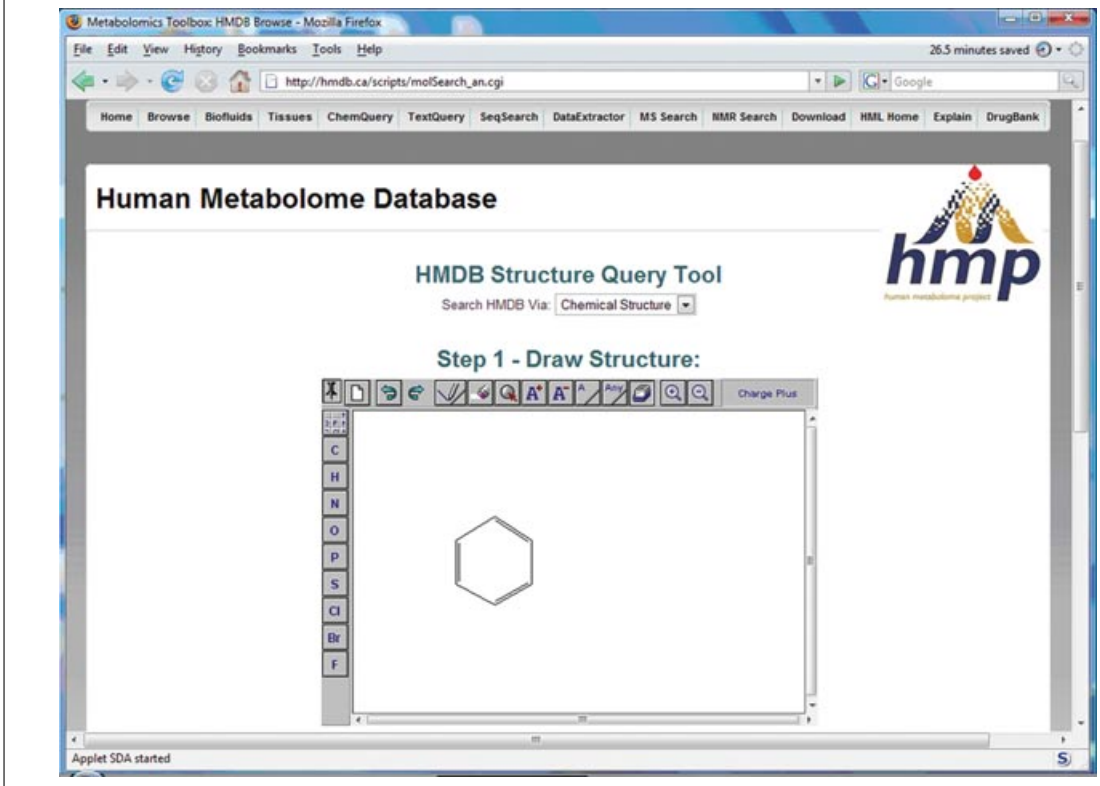


Figure 14.8.21 At this stage of the chemical structure drawing exercise, we have managed to draw a benzene ring.

of eleven aromatic ring structures should appear. A benzene ring should appear on the upper left corner of this pane. Click anywhere on the benzene ring to copy this structure and click just to the left of center in the structure drawing applet window to paste the six-carbon ring structure. The chemical structure drawing window should now appear as shown in Figure 14.8.21.

If a mistake is made, the user can easily undo the previous action by clicking on the undo button (cyan arrow curving up to the left, third button from the left). To the left of this button is a button that looks like a blank sheet of paper with the corner folded down. This button allows the user to start over and clear the drawing area. When using the chemical structure templates, two elements from different templates are usually joined at the elements to form a bond. However, it is possible to inadvertently join an element from one template structure to the middle of a bond of another template structure by clicking on a bond rather than an element (or vice versa). This type of structure is unrealistic and undesirable.

5. At this point, the user will draw in the rest of the atoms by hand using the vertical column of element buttons (C, H, N, O, P, S, etc.) on the left side of the drawing applet. Start with the oxygen atoms; click on the **O** to start adding oxygens. Add the first oxygen by clicking at a 45° angle above and to the left of the carbon at position 6. To draw the bond between the oxygen and carbon, mouse over the oxygen; a box should appear around the oxygen. Click and drag toward the carbon at position 6 until a box outlines this carbon. Upon release of the mouse button, a bond is drawn. Similarly, click at a 45° angle below and to the left of the carbon at position 5. Draw the bond as described above. Your drawing should look like the drawing shown in Figure 14.8.22. In a similar fashion, we will now add two carbons (by clicking on the **C** button) branching off from the carbon at position 3 and a nitrogen atom at the end of the two-carbon chain. The applet looks after the details in terms of the number of

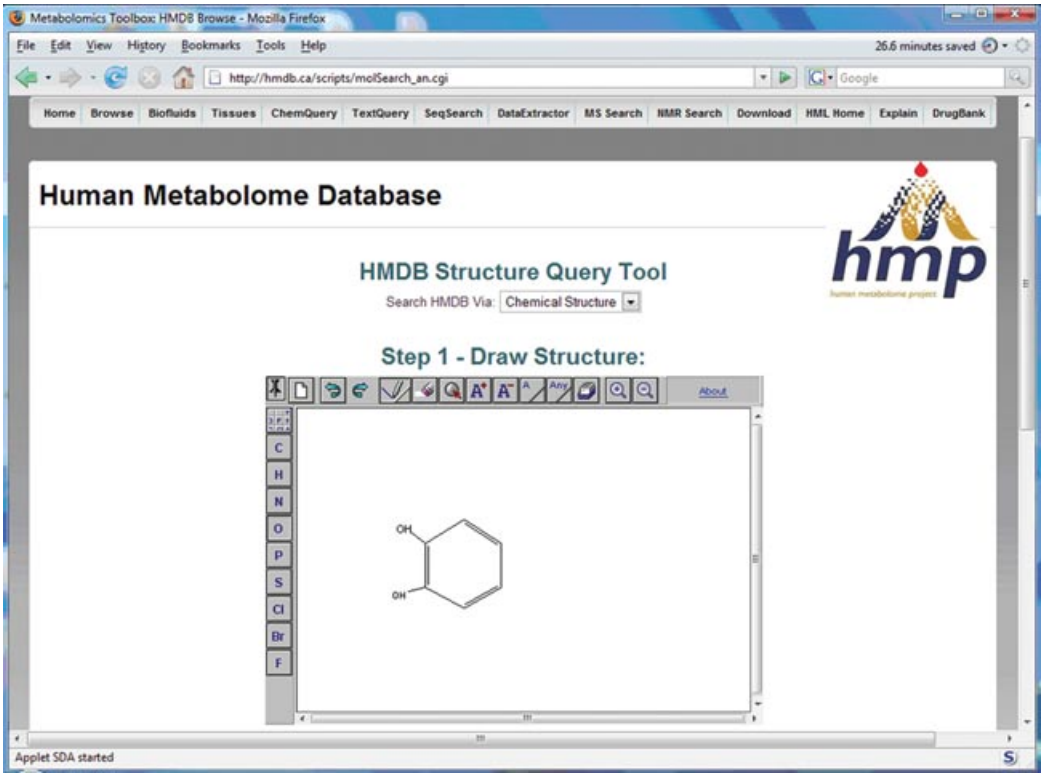


Figure 14.8.22 Our dopamine drawing is beginning to take shape. At this point, we have added two oxygens to the benzene carbons at positions 5 and 6.

appropriate hydrogen atoms. The finished chemical drawing should appear as shown in Figure 14.8.23.

6. Scroll down the ChemQuery page and click on the button labeled **CLICK TO CONVERT TO MOL FILE**. Clicking on this button will generate a MOL file that should automatically appear in the text box below the button (Fig. 14.8.24).

   The newly created MOL file text data can be copied and pasted into a text editor and stored for future use. The file conversion program Babel converts the MOL file data to a SMILES string that unambiguously describes the chemical structure drawing that was completed in this exercise.

7. Scroll down the ChemQuery page to the bottom and click on the button labeled **CLICK TO SUBMIT QUERY**. Within a few seconds, the search results window should replace the query page. The results page provides a ranked list of small molecules with similar chemical structures, with the best matches appearing near the top of the page. The results table looks very much like the HMDB Browser table. One notable exception is the assignment of a score that appears below the HMDB ID in the results table, with higher scores indicating better matches. Dopamine is a precursor to epinephrine (adrenaline) and norepinephrine (noradrenaline), both of which appear on the list of chemically similar structures (Fig. 14.8.25).

   This action takes the newly generated MOL file and converts it to a SMILES string. This SMILES string is then used as the query against a database of SMILES strings for all metabolites in the HMDB. This search is equivalent to a "text search" or a simple sequence alignment (such as BLAST). The ChemQuery search engine essentially identifies similar chemical structures by looking for shared SMILES substrings. A heuristic scoring method is used to prioritize and rank the substring matches and generate an overall matching score.

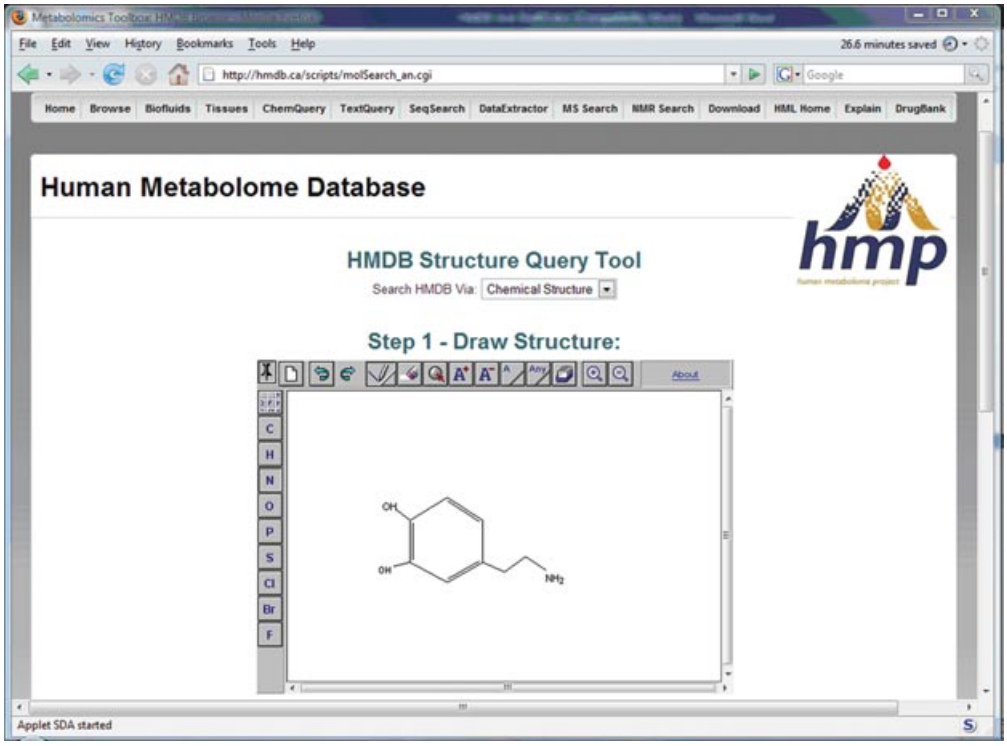

Metabolomics Toolbox: HMDB Browse - Mozilla Firefox
File Edit View History Bookmarks Tools Help
26.6 minutes saved
http://hmdb.ca/scripts/molSearch_an.cgi
Google
Home Browse Biofluids Tissues ChemQuery TextQuery SeqSearch DataExtractor MS Search NMR Search Download HML Home Explain DrugBank
Human Metabolome Database
hmp
HMDB Structure Query Tool
Search HMDB Via: Chemical Structure
Step 1 - Draw Structure:
About
C H N O P S Cl Br F
OH
OH
NH2
Applet SDA started

Figure 14.8.23 At this stage, the completed dopamine drawing is shown.

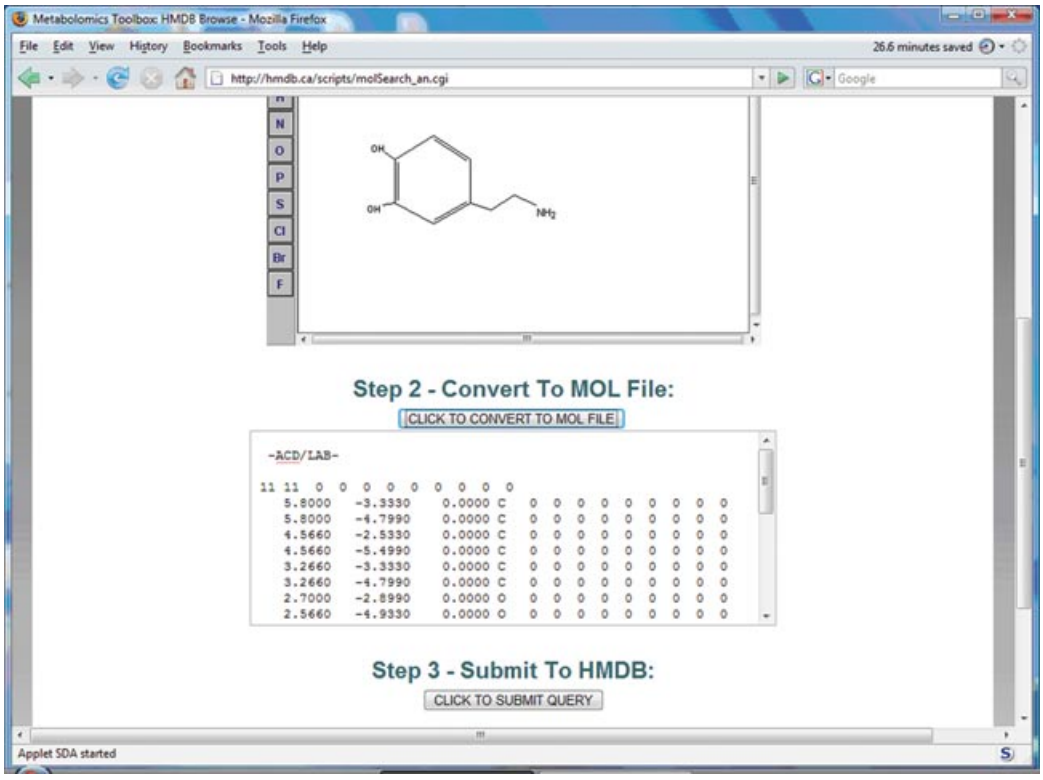

Metabolomics Toolbox: HMDB Browse - Mozilla Firefox
File Edit View History Bookmarks Tools Help
26.6 minutes saved
http://hmdb.ca/scripts/molSearch_an.cgi
Google
N O P S Cl Br F
OH
OH
NH2
Step 2 - Convert To MOL File:
CLICK TO CONVERT TO MOL FILE
-ACD/LAB-
11 11 0 0 0 0 0 0 0 0 0
5.8000 -3.3330 0.0000 C 0 0 0 0 0 0 0 0 0
5.8000 -4.7990 0.0000 C 0 0 0 0 0 0 0 0 0
4.5660 -2.5330 0.0000 C 0 0 0 0 0 0 0 0 0
4.5660 -5.4990 0.0000 C 0 0 0 0 0 0 0 0 0
3.2660 -3.3330 0.0000 C 0 0 0 0 0 0 0 0 0
3.2660 -4.7990 0.0000 C 0 0 0 0 0 0 0 0 0
2.7000 -2.8990 0.0000 O 0 0 0 0 0 0 0 0 0
2.5660 -4.9330 0.0000 O 0 0 0 0 0 0 0 0 0
Step 3 - Submit To HMDB:
CLICK TO SUBMIT QUERY
Applet SDA started

Figure 14.8.24 The MOL file text is automatically copied to the window below the CONVERT TO MOL FILE button.

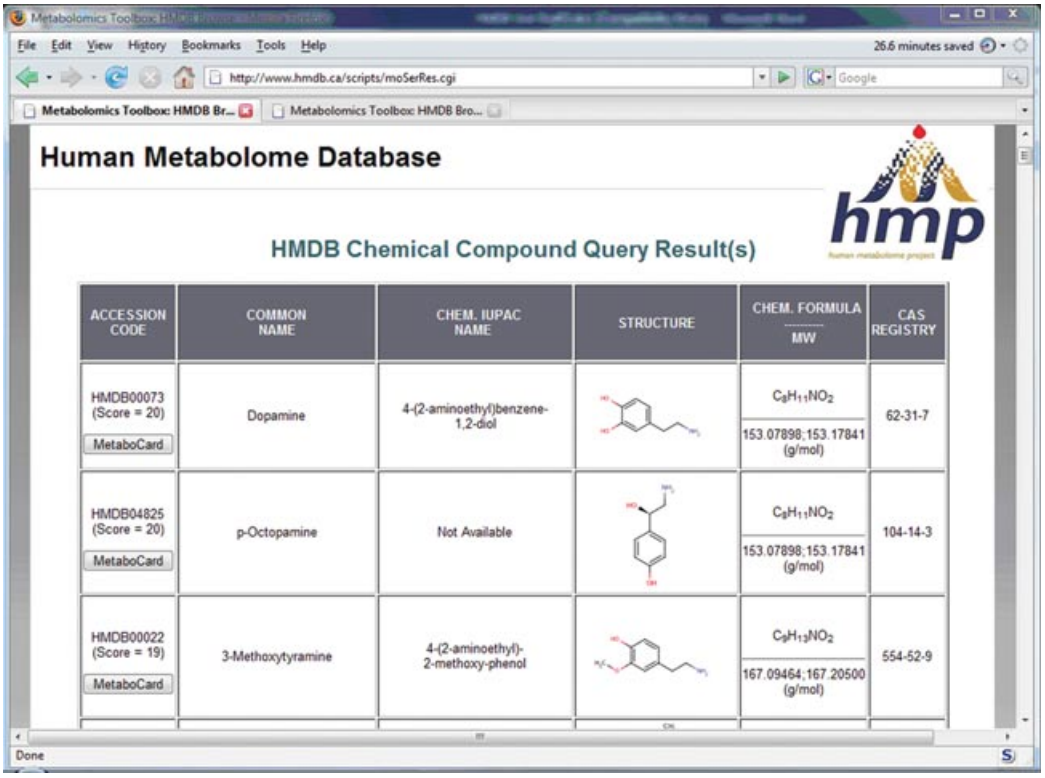


Figure 14.8.25 Here is the chemical similar search result obtained for our hand-drawn example.

Using the SMILES string option from ChemQuery

This protocol has described the use of a chemical drawing tool to draw a chemical structure and the use of this structure to search the Human Metabolome Database. However, the user can also use ChemQuery to search by SMILES string (Weininger, 1988) as mentioned in step 2 above.

8. Return to the ChemQuery window by clicking on the **ChemQuery** hyperlink on the HMDB menu. To search by SMILES string, select "SMILES String" (the last menu item) in the **SEARCH HMDB Via** pull-down menu. A new window should appear with a pull-down menu and a text box. Leave the pull-down menu at SMILES String and copy and paste a known SMILES string into the text box. In this case, the user enters the SMILES string for dopamine `(NCCC1=CC=C(O)C(O)=C1)` and clicks on the Search button to obtain the result. It is clear from this example that searching by SMILES string provides a much quicker, more convenient method of searching for similar chemical structures than manually drawing a structure.

9. Chemical structure similarity searches may also be performed from the HMDB's MetaboCards. At the upper right-hand corner of every MetaboCard (Fig. 14.8.26), directly below the Human Metabolome Project (HMP) logo, is a button labeled **Show Similar Structure(s)**. Clicking on this button should open a new window displaying a table of small molecules with similar chemical structures (Fig. 14.8.27). With this search method, users can freely browse the HMDB for chemical structures of interest and then use this feature to compare their structure with other related structures.

Both "Show Similar Structures" and ChemQuery use a locally developed SMILES string comparison method to identify related structures and to perform structure similarity searches. All structures are converted to SMILES strings, and a substring matching program (similar to BLAST) is used to identify similar structures. The scoring scheme is

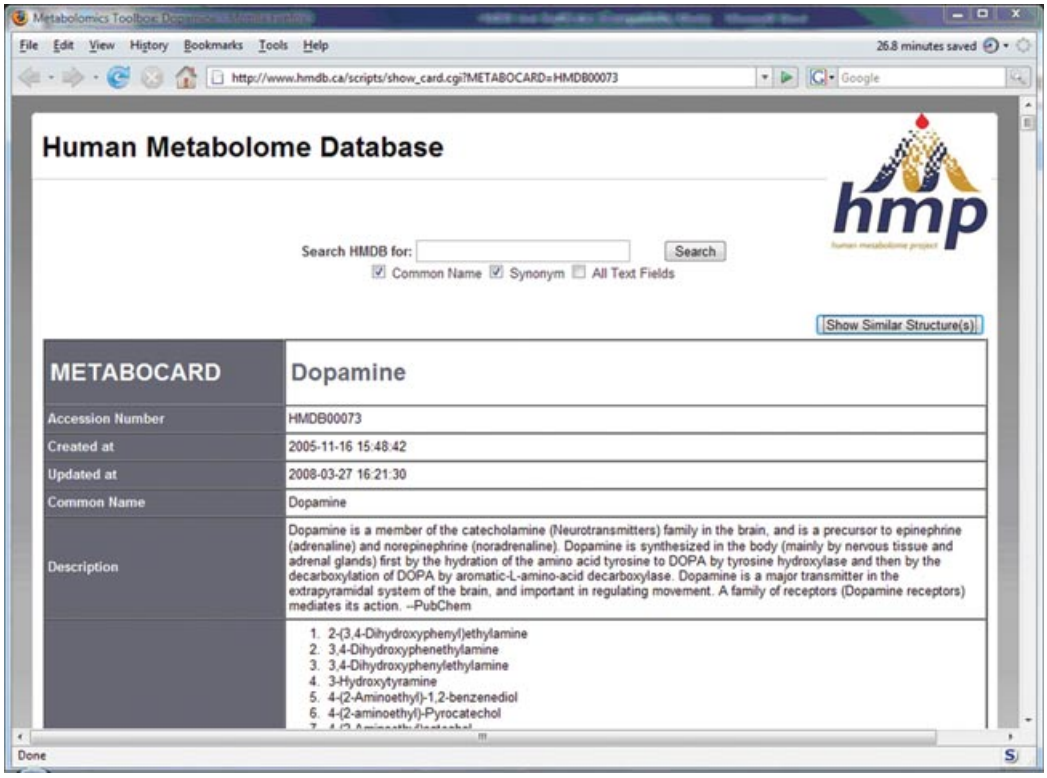

Metabolomics Toolbox
File Edit View History Bookmarks Tools Help
26.8 minutes saved
http://www.hmdb.ca/scripts/show_card.cgi?METABOCARD=HMDB00073
Google
Human Metabolome Database
hmp
Search HMDB for:
Search
Common Name Synonym All Text Fields
Show Similar Structure(s)
METABOCARD
Dopamine
Accession Number
HMDB00073
Created at
2005-11-16 15:48:42
Updated at
2008-03-27 16:21:30
Common Name
Dopamine
Description
Dopamine is a member of the catecholamine (Neurotransmitters) family in the brain, and is a precursor to epinephrine (adrenaline) and norepinephrine (noradrenaline). Dopamine is synthesized in the body (mainly by nervous tissue and adrenal glands) first by the hydration of the amino acid tyrosine to DOPA by tyrosine hydroxylase and then by the decarboxylation of DOPA by aromatic-L-amino-acid decarboxylase. Dopamine is a major transmitter in the extrapyramidal system of the brain, and important in regulating movement. A family of receptors (Dopamine receptors) mediates its action. --PubChem
1. 2-(3,4-Dihydroxyphenyl)ethylamine
2. 3,4-Dihydroxyphenethylamine
3. 3,4-Dihydroxyphenylethylamine
4. 3-Hydroxytyramine
5. 4-(2-Aminoethyl)-1,2-benzenediol
6. 4-(2-aminoethyl)-Pyrocatechol
Done

Figure 14.8.26 The Show Similar Structure(s) button appears at the top right of each MetaboCard in the HMDB.

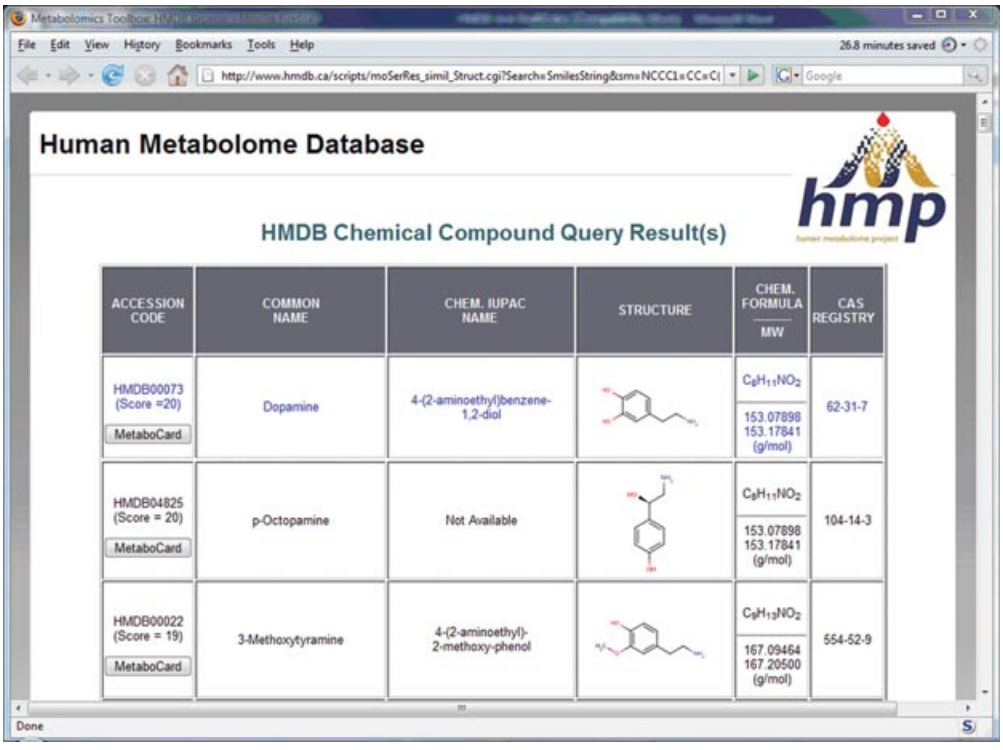


Figure 14.8.27 Here are the results obtained using the Show Similar Structure(s) button from the dopamine MetaboCard.

based simply on the number of character matches for the longest matching substring. A more robust substructure matching algorithm based on subgraph isomorphisms and the Tamimoto index is currently under development.

BASIC PROTOCOL 3

METABOLITE IDENTIFICATION VIA SPECTRAL MATCHING

This protocol describes how users may browse, search, and query the spectral databases in the HMDB. One of the key challenges in metabolomics is being able to identify metabolites from NMR, LC-MS, or GC-MS spectra collected from biofluids and tissues. Typically the spectra collected from these biological samples contain dozens to thousands of peaks (depending on the technology, the biosample, and the separation methods used). In some cases, the compound of interest has been purified, and so the spectrum may only contain a small number of peaks. Regardless of whether the NMR/GC-MS/LC-MS spectra are collected from a mixture or from a purified preparation, the best way of identifying an unknown compound or mixture of compounds is through comparison of the sample's peak positions (chemical shift, retention time, *m*/*z* value) to a library of standard or reference spectra (Wishart, 2007). In the case of compound identification via NMR, MS/MS, or GC-MS methods, typically one must match multiple peaks and peak patterns to confirm the existence or identity of a compound. In the case of compound identification by high-resolution MS methods (such as FT-MS or OrbiTrap) it is sometimes sufficient to identify a compound by matching a single mass peak (the parent ion) and a retention time.

While a number of spectral libraries do exist, including NMRShiftDB (Steinbeck et al., 2003), Spectral Database for Organic Compounds (SDBS; *http://riodb01.ibase.aist.go.jp/sdbs/*), Golm Metabolome Database (Kopka et al., 2005), and the NIST Spectral Database (*http://www.nist.gov/srd/nist1a.htm*; Ausloos et al., 1999), many contain spectra collected in organic solvents (for NMR) or are mostly populated with spectra from compounds that are not metabolites or, at least, not mammalian metabolites. One of the strengths of the HMDB is the fact that it contains spectra of hundreds of common metabolites that were collected in aqueous conditions (especially for NMR) under controlled and clearly defined conditions. The HMDB NMR libraries contain 722 ^{1}H experimental NMR spectra, 709 ^{13}C HSQC NMR experimental spectra, 2515 ^{1}H predicted NMR spectra, and 2511 ^{13}C predicted NMR spectra. The HMDB GC-MS library contains 311 EI spectra with retention times corresponding to 281 metabolites and 30 TMS-derivitization variants. The HMDB MS/MS library contains 2137 MS/MS spectra collected on a Waters Quattro LC triple quadrupole mass spectrometer from 667 compounds at three different collision energies. Protocols describing the sample collection conditions and parameters are available at *http://www.metabolomics.ca/News/sops.htm*. Brief synopses of the data collection conditions for each metabolite are also available in the corresponding compound's MetaboCard.

The HMDB's reference spectra may be viewed within each MetaboCard (see Basic Protocol 1) or they may be viewed and searched using the corresponding GC-MS Search, MS-MS Search, and NMR Search facilities within the HMDB. These spectral search tools support querying via compound names (common name), synonyms, molecular weight range, chemical formula and—most importantly—by spectral peak positions. In particular, the HMDB's spectral matching tools allow users to identify compounds from NMR, GC-MS, or LC-MS spectra collected from either pure extracts or complex mixtures.

Necessary Resources

Hardware

Computer with Internet access

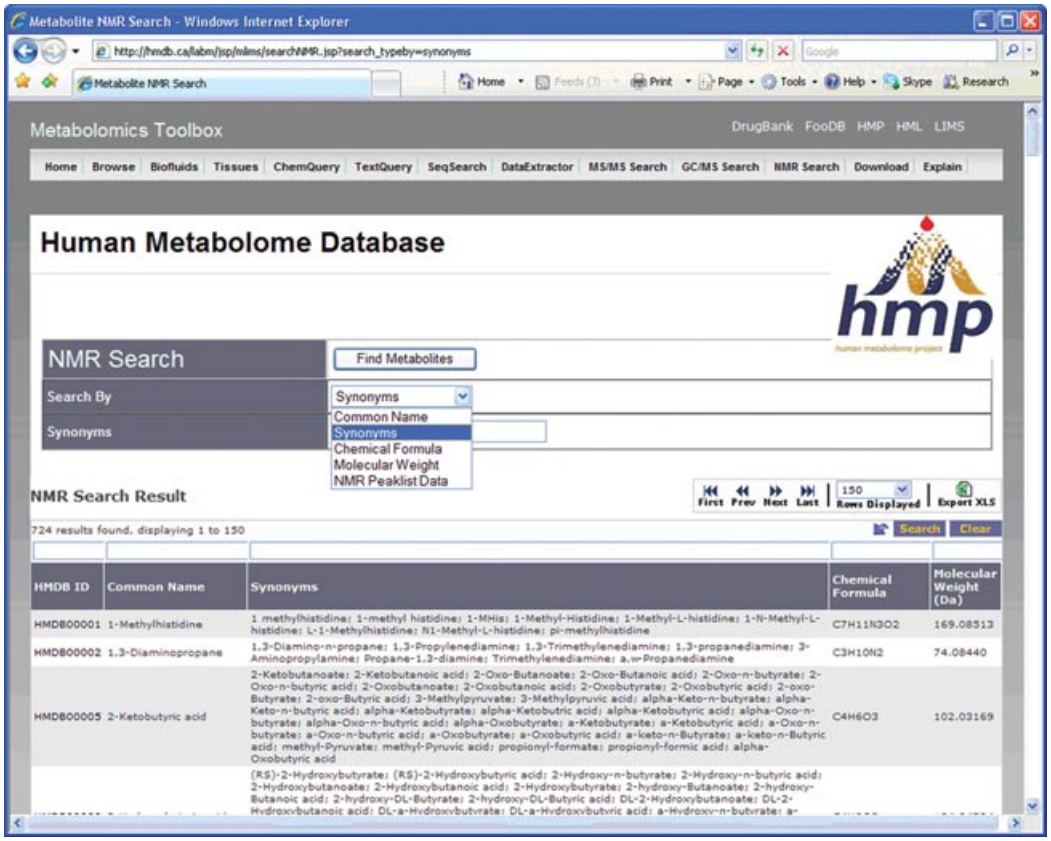


Figure 14.8.28 The spectral search pages (NMR, MS/MS, and GC-MS) include a convenient drop-down menu that allows users to search by Common Name, Synonyms, Chemical Formula, Molecular Weight, or respective peak list.

Software

An up-to-date Web browser, such as Internet Explorer (*http://www.microsoft.com/ie/*), Firefox (*http://www.mozilla.com/*), Netscape (*http://browser.netscape.com/*), Opera (*http://www.opera.com/*), or Safari (*http://www.apple.com/safari/*). The Web browser must be capable of handling Java applets (i.e., equipped with a recent version of the Java interpreter).

Files

None

Browsing and searching the HMDB's spectral databases

The HMDB spectral database search pages (NMR, MS/MS, and GC/MS) share a common user interface. Each page has a pull-down menu labeled **Search By** with the following five options: "Common Name," "Synonyms," "Chemical Formula," "Molecular Weight," and "Peaklist Data" (NMR, MS/MS, or GC/MS). This convenient interface allows the user to search all of the NMR, MS/MS, or GC/MS available spectra by common name, synonym, chemical formula, molecular weight, or peak list. As an example, the interface for the NMR Search page is shown in Figure 14.8.28.

Single compound identification via NMR search

1. Open your local Web browser and go to the HMDB home page at *http://hmdb.ca/.*

   The HMDB home page should be visible as should the light gray menu bar located near the top of the page with fourteen clickable links: Home, Browse, Biofluids, Tissues, ChemQuery, TextQuery, SeqSearch, DataExtractor, MS/MS Search, MS Search, GC/MS Search, NMR Search, Download, and Explain.

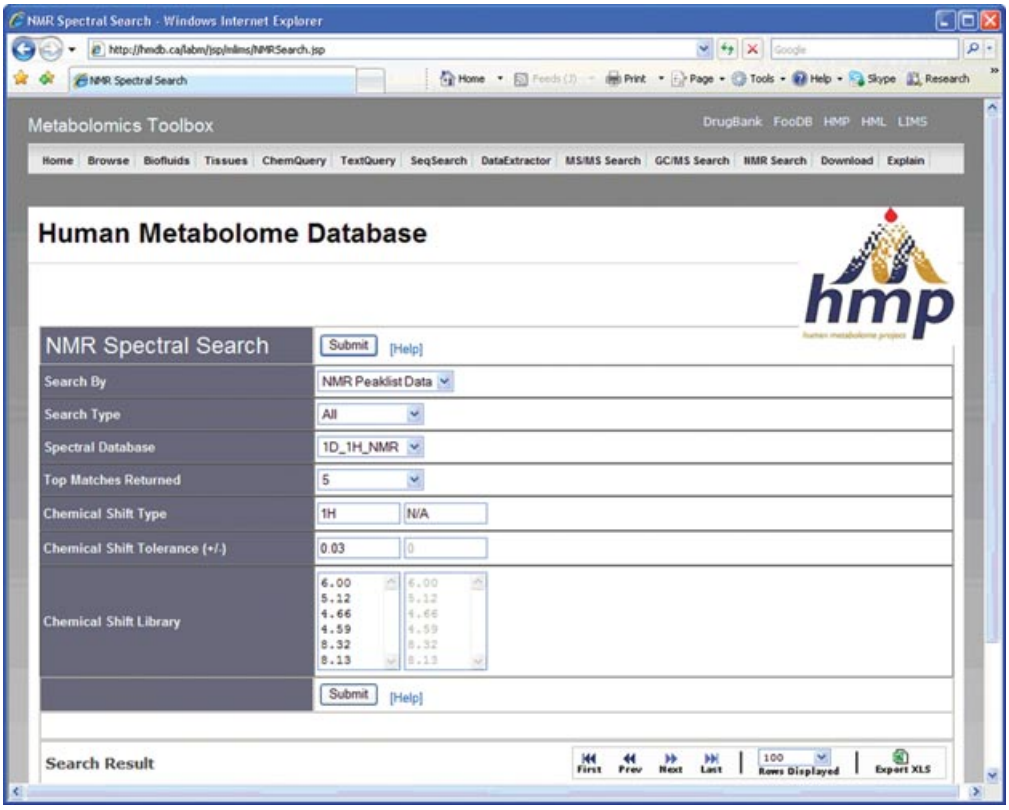


Figure 14.8.29 The NMR Search page allows users to search for a variety of NMR spectral types (1D ^{1}H, 1D ^{13}C, 2D HSQC, and 2D TOCSY).

2. Click on the **NMR Search** link (third from the right). After a few seconds the NMR default search page should appear as in Figure 14.8.28. Click on the pull-down menu to the right of **Search By** and select NMR Peaklist Data. After a few seconds, a window should appear with four pull-down tabs as well as two text boxes that accept numerical input for **Chemical Shift Tolerance (±)** and **Chemical Shift Library** (Fig. 14.8.29). The second pull-down menu (**Search Type**) allows users to select the type of NMR data (All, Experimental, or Predicted). The third pull-down menu (**Spectral Database**) allows users to select the spectral database to be searched (1D ^{1}H NMR, 1D ^{13}C NMR, 2D HSQC, or 2D TOCSY). The fourth pull-down menu (**Top Matches Returned**) allows users to select the number of matches to be displayed (5, 10, 20, or 100). In the first text box (**Chemical Shift Tolerance**), users enter a number representing how tightly they want the input peak list to match the peaks in the database. Lower numbers specify tighter matches while higher numbers specify looser matches. In the second text box (**Chemical Shift Library**), users enter all of the peaks that they can read from their NMR spectrum.

Entering some experimental NMR data

This particular part of the protocol will use the NMR Search link to take experimental NMR data and look for molecules with matching NMR peaks. Therefore, the user should leave the following three pull-down menus with their default selections (**Search Type**, **Spectral Databases**, and **Top Matches Returned**). It is important to note that the **Search By** pull-down menu must be set to "NMR Peaklist Data."

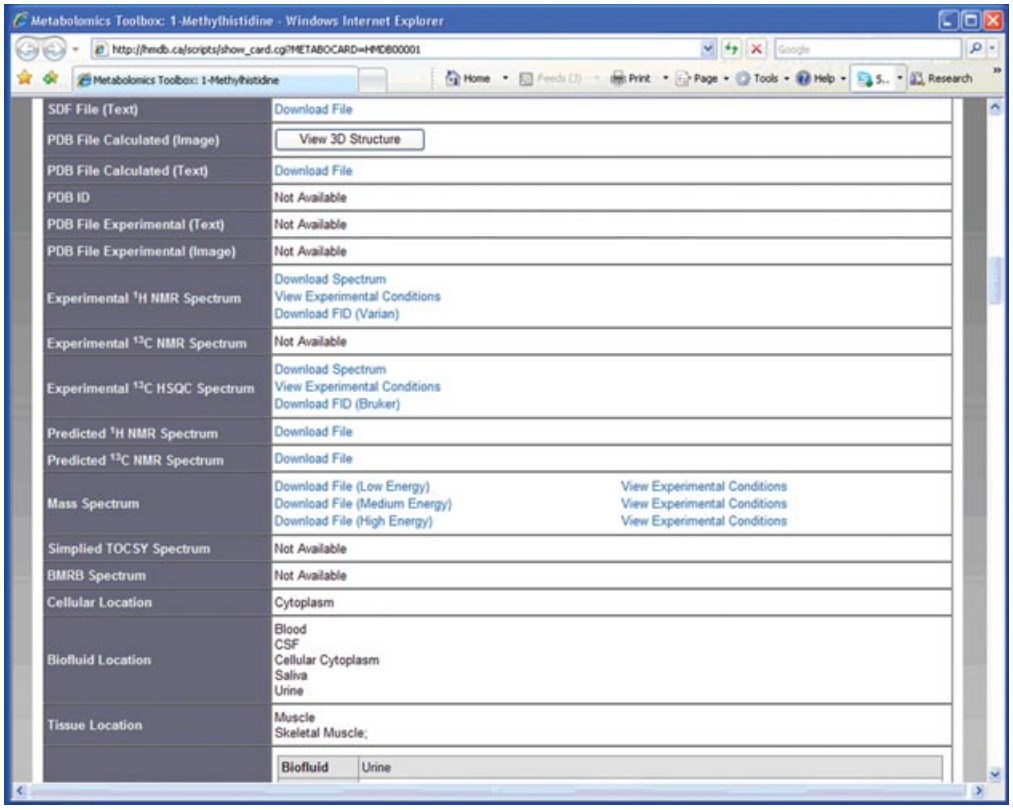

Metabolomics Toolbox: 1-Methylhistidine - Windows Internet Explorer
http://hmdb.ca/scripts/show_card.cgi?METABOCARD=HMDB00001
Metabolomics Toolbox: 1-Methylhistidine
SDF File (Text) Download File
PDB File Calculated (Image) View 3D Structure
PDB File Calculated (Text) Download File
PDB ID Not Available
PDB File Experimental (Text) Not Available
PDB File Experimental (Image) Not Available
Experimental 1H NMR Spectrum Download Spectrum View Experimental Conditions Download FID (Varian)
Experimental 13C NMR Spectrum Not Available
Experimental 13C HSQC Spectrum Download Spectrum View Experimental Conditions Download FID (Bruker)
Predicted 1H NMR Spectrum Download File
Predicted 13C NMR Spectrum Download File
Mass Spectrum Download File (Low Energy) View Experimental Conditions Download File (Medium Energy) View Experimental Conditions Download File (High Energy) View Experimental Conditions
Simplied TOCSY Spectrum Not Available
BMRB Spectrum Not Available
Cellular Location Cytoplasm
Biofluid Location Blood CSF Cellular Cytoplasm Saliva Urine
Tissue Location Muscle Skeletal Muscle;
Biofluid Urine

Figure 14.8.30 As you scroll down on the MetaboCard for 1-methylhistidine, the Experimental ^{1}H NMR Spectrum field will appear as shown.

3. For this example, we will use the Experimental ^{1}H NMR spectral data for 1-methylhistidine. In a separate browser window or tab, go to the HMDB home page (*http://hmdb.ca*). In the text search box near the top of the home page, enter `1-methylhistidine`. Click on the 1-methylhistidine hyperlink to go to the 1-methylhistidine MetaboCard. Scroll down the page to the **Experimental ^{1}H NMR Spectrum** field (Fig. 14.8.30). Click on the **Download Spectrum** link to view the spectrum in a new browser window (Fig. 14.8.31). This page provides the experimental proton NMR data about 1-methylhistidine, including an image of the NMR spectrum as well as a table of peaks. Scroll down to the table of peaks (Fig. 14.8.32) and enter the Chemical Shift values from the (ppm) column in your NMR search **Chemical Shift Library** text box. The values to be entered are `3.04`, `3.06`, `3.07`, `3.09`, `3.14`, `3.15`, `3.17`, `3.18`, `3.68`, `3.95`, `3.95`, `3.96`, `3.97`, `7.00`, and `7.68`.

   Each value must be entered on its own line with no non-numeric characters (e.g., whitespace).

4. The completed chemical shift library query data should appear as shown in Figure 14.8.33. After entering all of the values from the (ppm) column of the Table of Peaks, hit the Submit button.

5. Within a few seconds your search results should appear below the search area. As expected, the top hit should be 1-methylhistidine. The results appear as a table with several clickable hyperlinks including the HMDB ID, Peaklist, and Spectra (Fig. 14.8.34). Other fields are also displayed in this table including the name of the matching metabolites and the **Category** (Experimental or Predicted). If we click on the **Peaklist** hyperlink for 1-methylhistidine, a new window should open with the

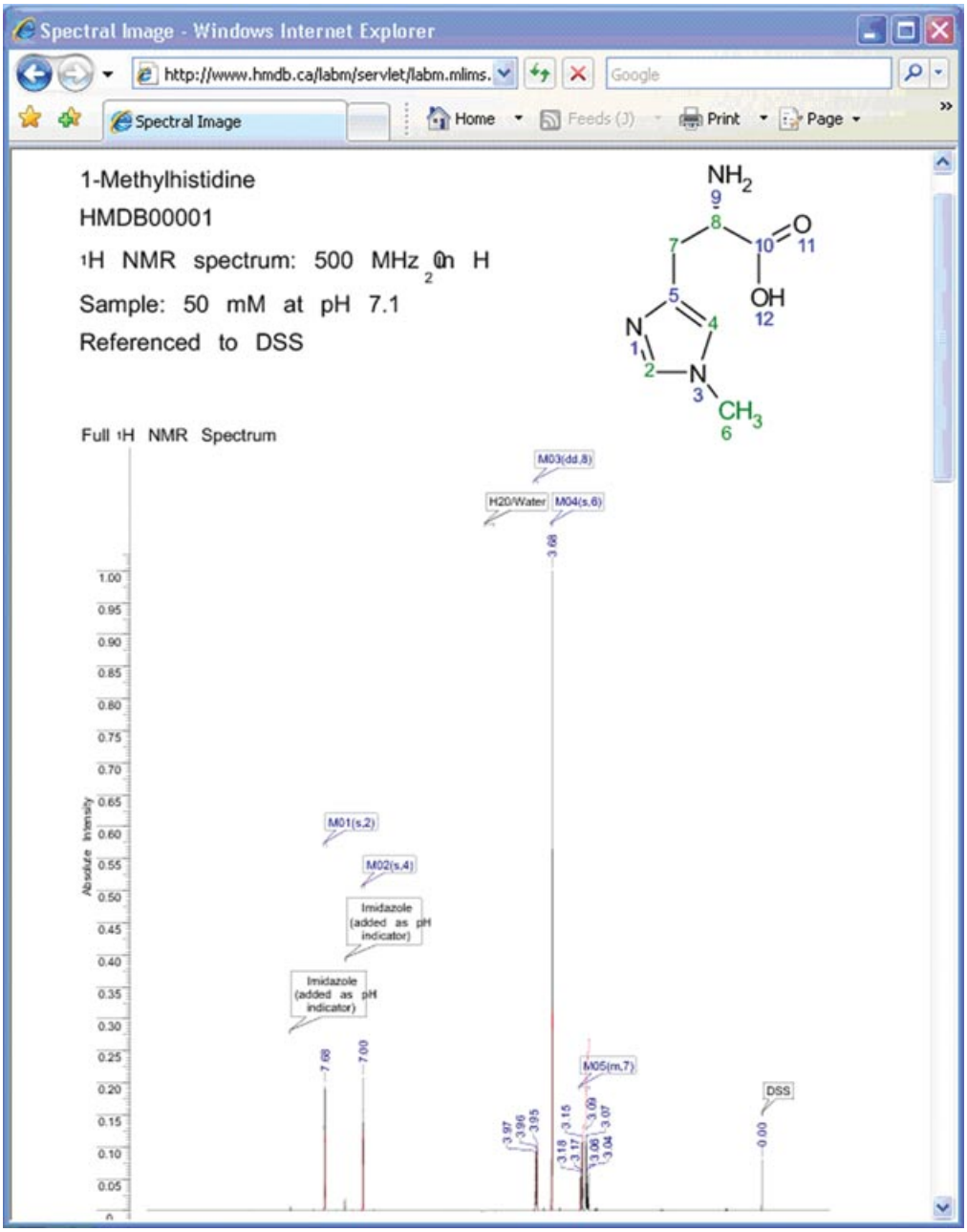


Figure 14.8.31 Here is the Experimental ^{1}H NMR Spectrum for 1-methylhistidine.

peak list data for this compound. An identical match to our input peak list has been found. If you view the peak lists for the other matches, note that only a portion of these peak lists match the query peak list.

Multiple compound or mixture ID via NMR search

6. In this example, the user will use NMR Search to identify compounds from a mixture of compounds. Scroll back up to the top of the NMR Search page and ensure that **Search By** "NMR Peaklist Data" is selected. In the second drop-down menu (**Search Type**), select "Experimental." Leave the third (**Spectral Database**) and fourth (**Top Matches Returned**) drop-down menus as well as the **Chemical Shift Tolerance** on the default settings. Select the numerical data in the **Chemical Shift Library** text box and hit the backspace or delete button to delete this text.

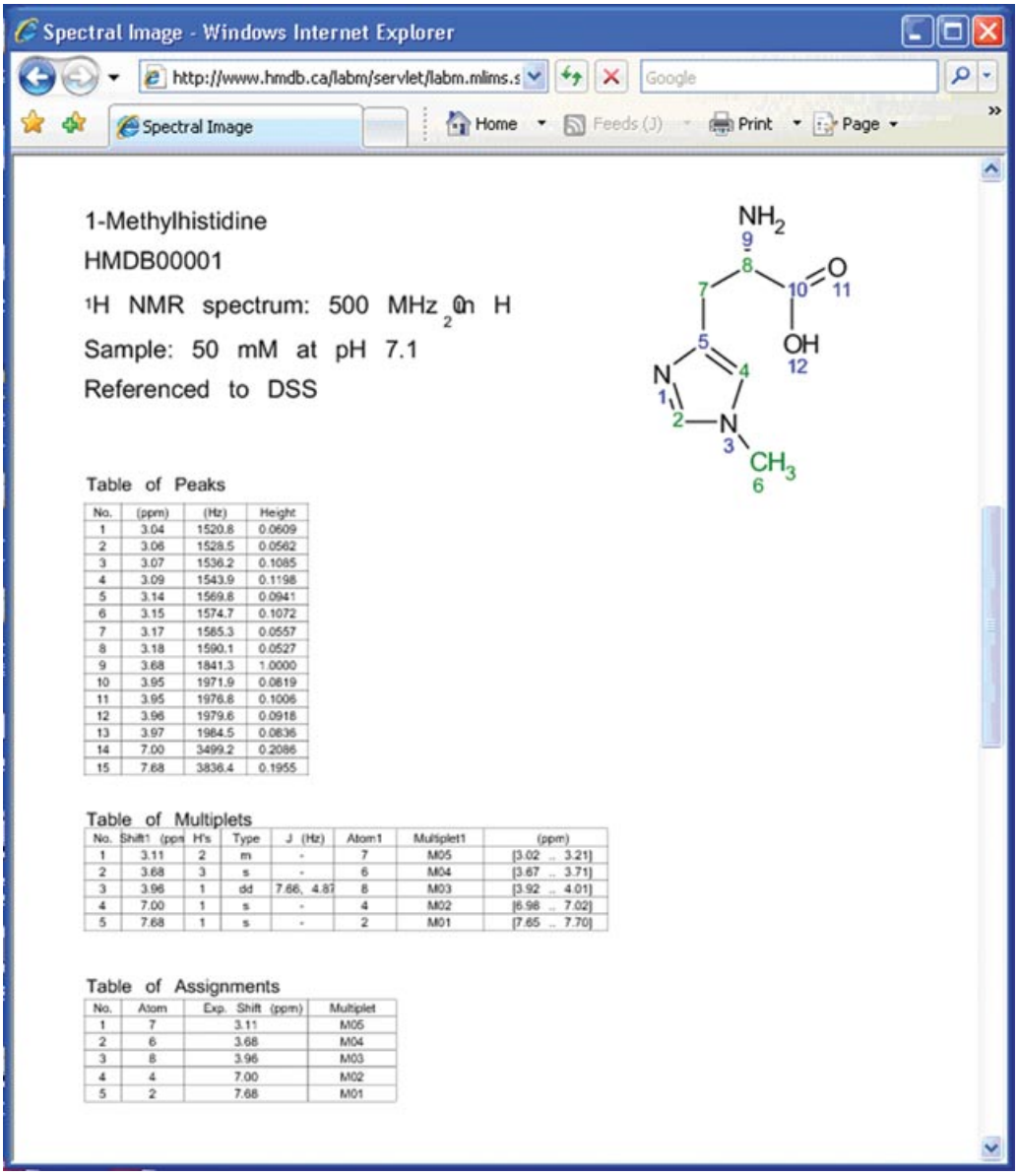


Figure 14.8.32 Here is the Table of Peaks for 1-methylhistidine.

With the cursor in this text box, type in the following spectral peak data representing experimental 1D ^{1}H NMR data for three metabolites: `0.804, 1.020, 1.206, 1.400, 1.642, 1.866, 2.071, 2.357, 3.663, 5.739, 2.68, 3.73, 4.14, 3.24, 3.05, 7.28, 7.40, 7.34, 7.40, 7.28, 3.665, 3.744, 7.349, 7.377,` and `7.412`. Enter each value on a separate line by hitting the Enter key after typing each value. The NMR Search window should appear as shown in Figure 14.8.35. Hit the Submit button to obtain the results.

7. After a few seconds, a results table should appear at the bottom of the NMR Search page. Scroll down the page to view these results. The results should appear as shown in Figure 14.8.36. The top three hits are testosterone, aspartame, and phenylacetylglycine. In this example, NMR Search has been used to successfully identify three compounds from a mixture of compounds. Note that the three compounds identified have relatively high scores (10/10, 8/8, and 5/5, respectively) as listed in the far right column. In the case of testosterone, ten peaks from the query peak list matched

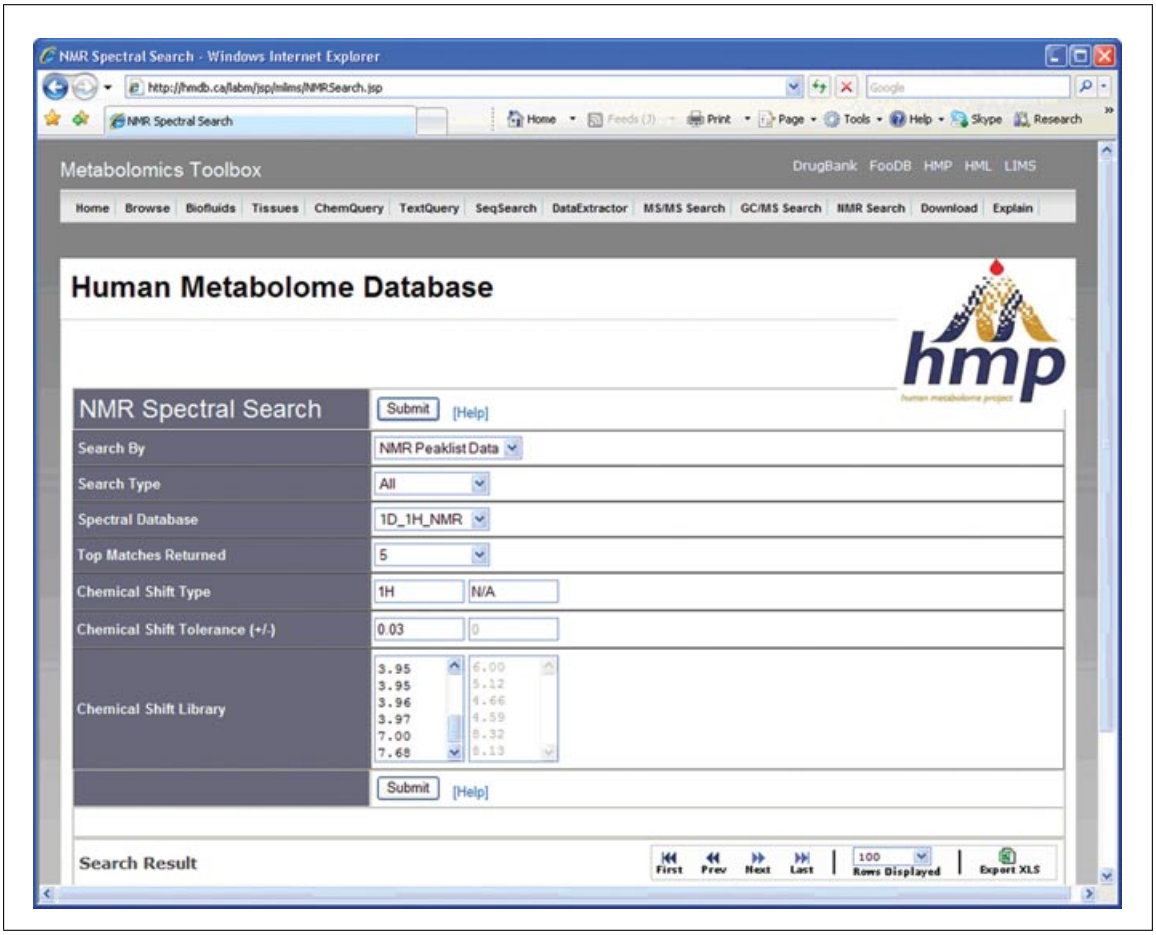


Figure 14.8.33 The completed chemical shift library query data for 1-methylhistidine should appear as shown.

the peak list for testosterone in the HMDB NMR spectral library. As in the single compound identification section of this protocol, the results table is sortable by column. To sort by column, simply click on the column heading hyperlink. Thus, the table is sortable by **HMDB ID**, **Name**, **Category** (Experimental or Predicted), and **Score**. The text boxes above each column heading allow users to search for a specific compound by **HMDB ID**, **Name**, **Category**, or **Score**. In the table, the following fields contain hyperlinks: **HMDB ID** and **Peaklist**, allowing for convenient access to the MetaboCard and peak list data. The user can navigate the results table using the convenient hyperlinked arrows on the top right of the table (**First**, **Previous**, **Next**, **Last**). The user can also choose to display 50 or 100 rows and can export the results table to Microsoft Excel using the hyperlinked **Export XLS** icon or text.

Compound identification via MS search

8. Scroll back up to the top of the NMR Search page and click on the MS/MS Search hyperlink on the menu bar just to the right of center. You should now see the MS/MS Search page. From this page, users can perform MS/MS searches, MS searches, peak list searches, and GC/MS searches. Click on the top pull-down menu to view the different MS search options.

9. From the top pull-down menu, click once on **MS Search**. Within a few seconds the MS Search page should appear as shown in Figure 14.8.37. Below the Find Metabolites button, the user can select which databases to search (**HMDB**, **Theoretical MS/MS**, **FooDB**, **DrugBank**, or **All Databases**). FooDB is the Food Component Database with over 1900 food components (*http://www.foodbs.org/foodb*)

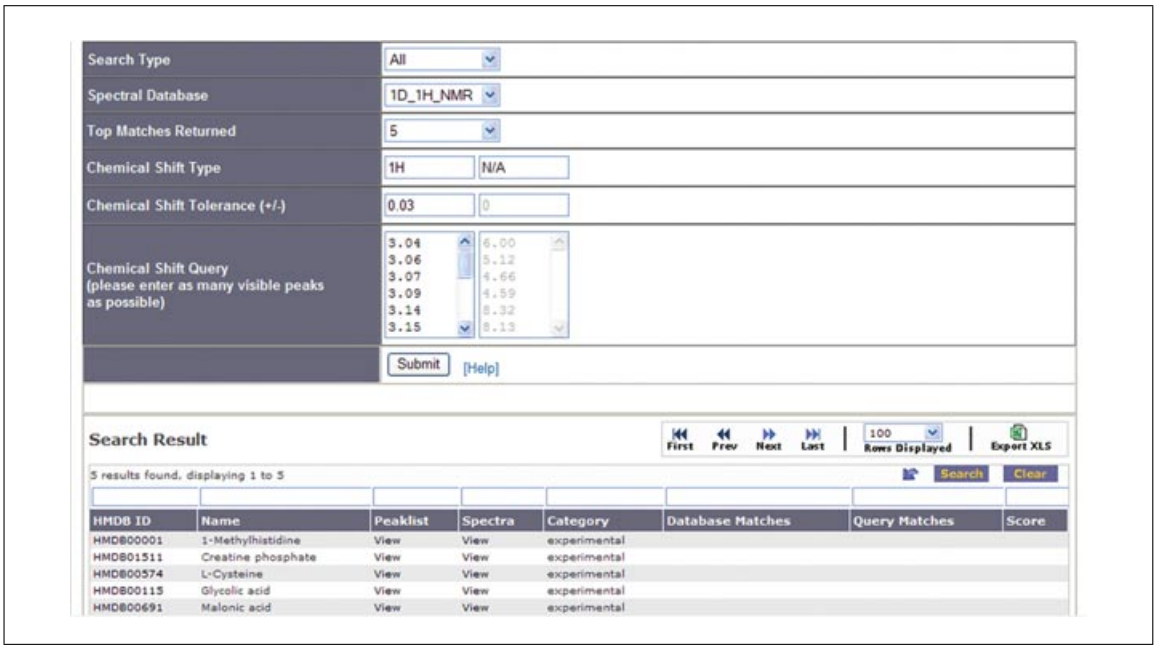


Figure 14.8.34 Here is the results table for the 1D ^{1}H NMR search using the peak list for 1-methylhistidine.

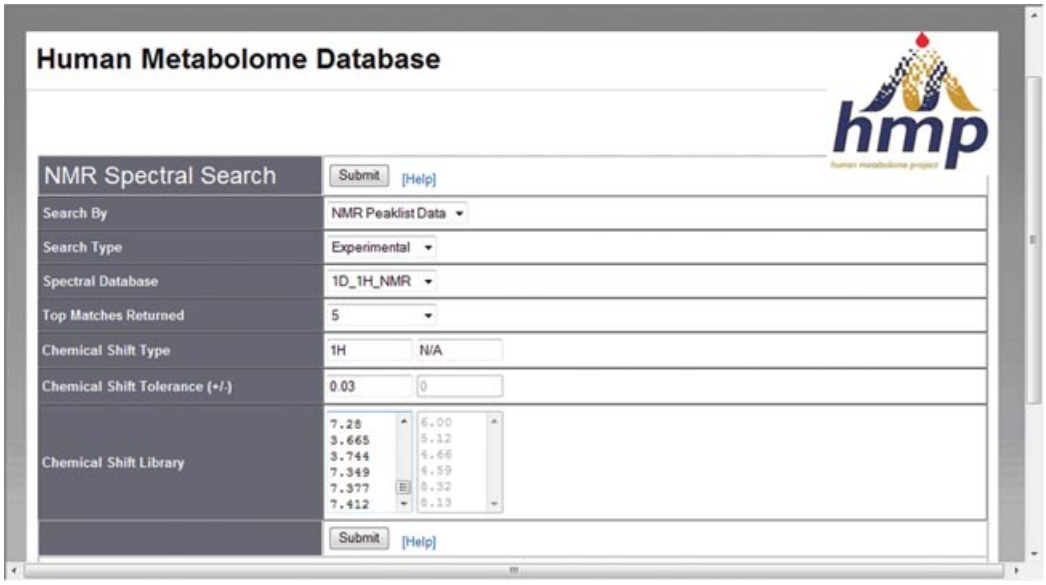


Figure 14.8.35 For the multiple compound identification example, the NMR Search window should appear as shown.

while DrugBank (*http://www.drugbank.ca/*) is a database with over 4700 drugs. Below the database field are two text boxes, one for the **MW of Parent Ion** and the other for the **MW Tolerance**. The user can type or copy/paste numerical data into these text boxes. The MW of Parent Ion represents the molecular weight in daltons of the unfragmented compound. The MW Tolerance field allows the user to specify the "stringency" of the search, with lower numbers specifying tighter matches to the query value. In this example, we will check off only the **HMDB database**, enter 129 in the **MW Parent Ion** text box, keep the default **MW Tolerance** value of 0.1 Da, and press the "Find Metabolites" button.

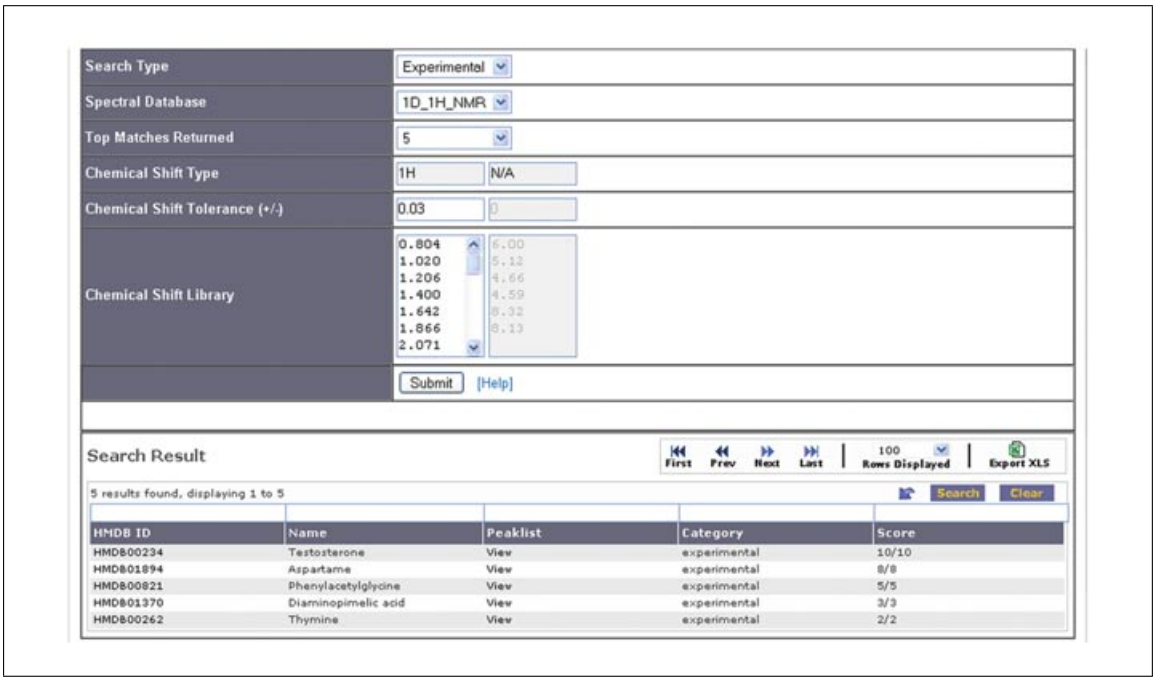


Figure 14.8.36 Of the five hits that appear in the results table, the top three represent the three compounds that make up the mixture of compounds.

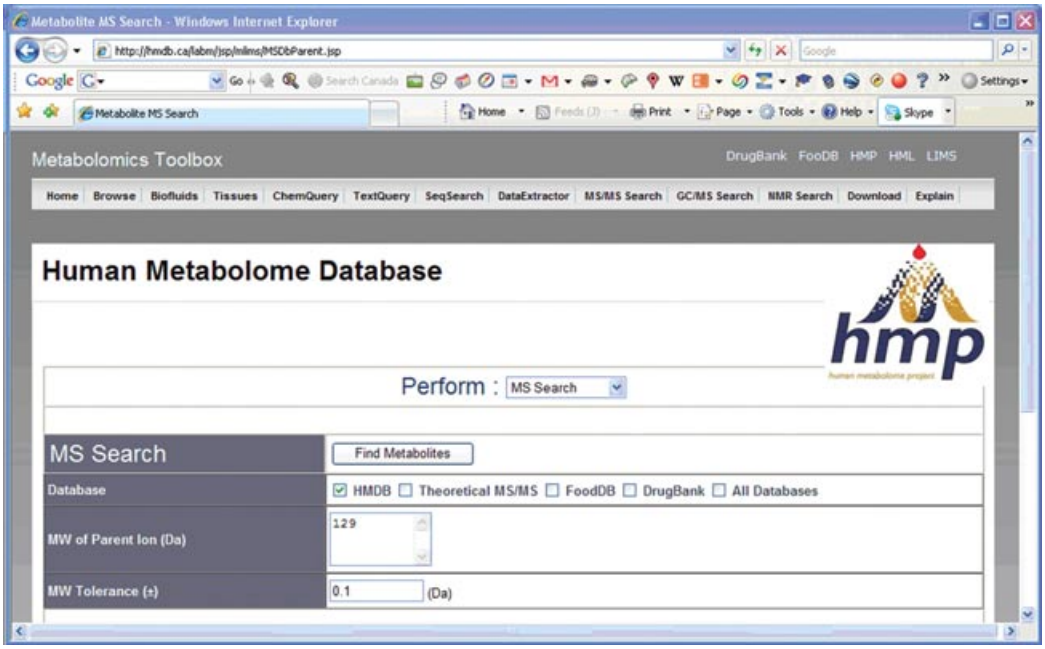


Figure 14.8.37 The MS Search page can be used to look for metabolites with matching molecular weight.

10. After a few seconds, the results should appear below the search area as a four-column table as shown in Figure 14.8.38. The four columns in this table are **Rank**, **HMDB ID**, **Name**, and **Isotopic MW**. The HMDB ID fields in this table are hyperlinked and clicking on one of these HMDB ID hyperlinks opens up the corresponding MetaboCard.

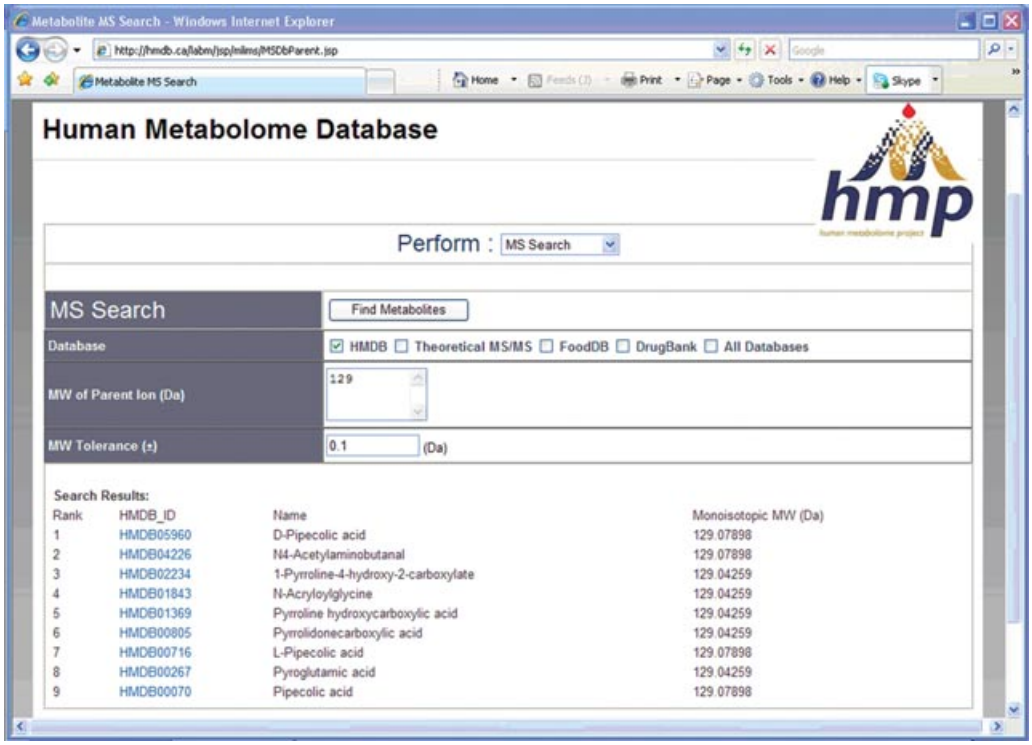


Figure 14.8.38 The MS Search results should appear as a four-column table with Rank, HMDB ID, Name, and Monoisotopic Molecular Weight.

Compound identification via MS/MS search

11. Now, the user will proceed to the MS/MS Search page by clicking on MS/MS Search from the top pull-down menu. A new browser window should open with the MS/MS Search page. Click on the pull-down menu to the right of **Search by** and select MS/MS Peaklist Data. A new browser window should appear with four text boxes (**m/z of Parent Ion**, **m/z Tolerance**, **Fragment Ion Tolerance**, and **Content of MS/MS Data File**) and four pull-down menus (**Search By**, **Instrument Type**, **CID Energy Level**, and **Ionization Mode**); a Browse button to the right of a text box should appear on the row marked **MS/MS Data File**. For the **Search By** pull-down menu, the user can choose between five different options ("Common Name," "Synonyms," "Chemical Formula," "Molecular Weight," or "MS/MS Peaklist Data"). For the **Instrument Type** pull-down menu, there are four different choices ("Triple_Quad," "QTOF," "FTMS," or "Ion_Trap"). For the **CID Energy Level** pull-down menu, there are four possible choices ("Low Energy," "Medium Energy," "High Energy," or "All"). For the **Ionization Mode** pull-down menu, users can choose between three options ("Negative," "Positive," or "N/A"). The Browse button allows a user to upload an MS/MS data file from the local computer or a network file server.

12. In this example, we will use the data for the small molecule aconitic acid. In a separate browser window or tab, open up the Human Metabolome Database home page (*http://hmdb.ca/*). In the text search box at the top of the home page, enter `aconitic acid` and hit the Submit button. In a few seconds, the text search results should appear with two hits, *cis*-Aconitic acid and *trans*-Aconitic acid. Click on the *cis*-Aconitic acid link to view its MetaboCard. Scroll down to the **Mass Spectrum** field and click on the **View Experimental Conditions** link to the right of the **Download File (Low Energy)** hyperlink. A new browser window will appear

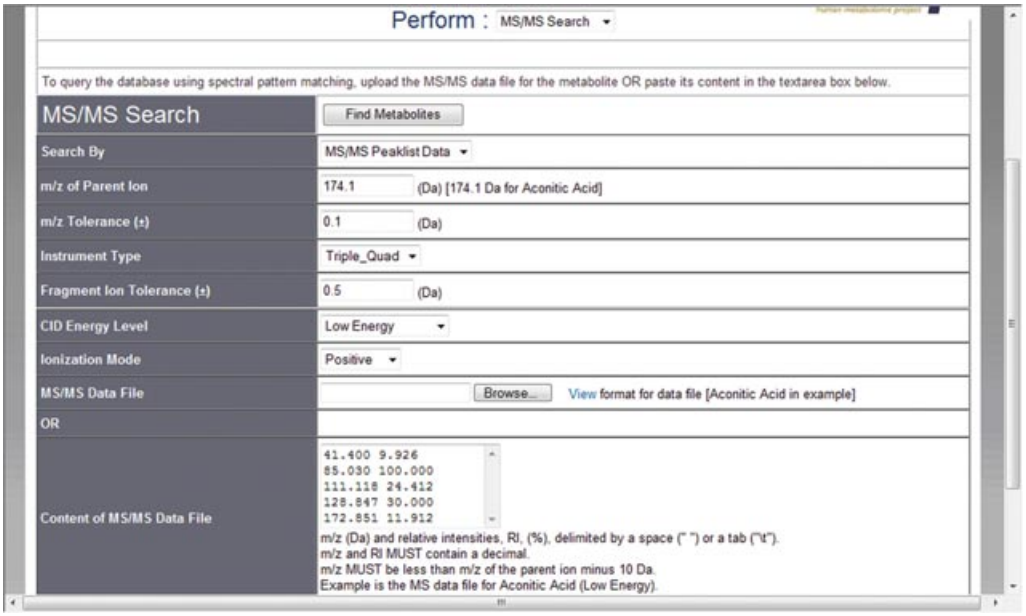

Perform : MS/MS Search
To query the database using spectral pattern matching, upload the MS/MS data file for the metabolite OR paste its content in the textarea box below.
MS/MS Search
Find Metabolites
Search By
MS/MS Peaklist Data
m/z of Parent Ion
174.1
(Da) [174.1 Da for Aconitic Acid]
m/z Tolerance (±)
0.1
(Da)
Instrument Type
Triple_Quad
Fragment Ion Tolerance (±)
0.5
(Da)
CID Energy Level
Low Energy
Ionization Mode
Positive
MS/MS Data File
Browse...
View format for data file [Aconitic Acid in example]
OR
Content of MS/MS Data File
41.400 9.926
85.030 100.000
111.118 24.412
128.847 30.000
172.851 11.912
m/z (Da) and relative intensities, RI, (%), delimited by a space (" ") or a tab ("t").
m/z and RI MUST contain a decimal.
m/z MUST be less than m/z of the parent ion minus 10 Da.
Example is the MS data file for Aconitic Acid (Low Energy).

Figure 14.8.39 The MS/MS Search page should appear as shown with all the fields as default settings except for the ionization mode and the Search By pull-down menu set at MS/MS Peaklist Data.

with details on the experimental conditions. Note the **Instrument Type** and the **Ionization Mode**; in this case, Quatro_QQQ or Triple Quad for the Instrument Type and positive for the Ionization Mode. With this information, return to the MS/MS Search page and use the defaults for m/z Parent Ion, m/z Tolerance, Instrument Type (default is Triple_Quad), Fragment Ion Tolerance, CID Energy Level (default is Low Energy), and the Content of MS/MS Data File (the default is aconitic acid). It is important to note that the **Search By** pull-down menu should be set to MS/MS Peaklist Data. The user just needs to change the **Ionization Mode** from Negative to Positive using the pull-down menu. The MS/MS Search page with the appropriate values and selections made should appear as shown in Figure 14.8.39. Click on the Find Metabolites button to launch the search.

13. In a few seconds, a new window should appear with the search results at the bottom of the page. The search results should appear as shown in Figure 14.8.40. The results appear in an eight-column table with the following columns: **Rank**, **HMDB_ID**, **Name**, **Fit(%)**, **RFit(%)**, **Purity(%)**, **Energy Level**, and **Data**. The HMDB_ID column contains hyperlinks to the MetaboCards for the matching HMDB compounds, while the Data column contains two separate hyperlinks to a matching **Peaklist** and **Spectrum**. Note that there are two compounds (*trans*-Aconitic acid and *cis*-Aconitic acid) with RFit(%) values of 1, indicating two identical matches. An RFit(%) value of 1 indicates a perfect match. They have identical masses since they are *cis-trans* stereoisomers.

Compound identification via GC/MS search

14. In this last part of the protocol, the user will learn more about the GC/MS Search page. From the MS/MS Search page, scroll back up to the top of the page and click on the top pull-down menu to the right of **Perform**. Click on the GC/MS hyperlink to open the GC/MS Search page. Click on the **Search By** pull-down menu and select GC/MS Peaklist Data to open a new browser window. This page is quite a bit simpler than the MS/MS Search page. Except for the **Perform** and **Search By** pull-down menus, there are no pull-down menus on this search page. However, there are six text boxes

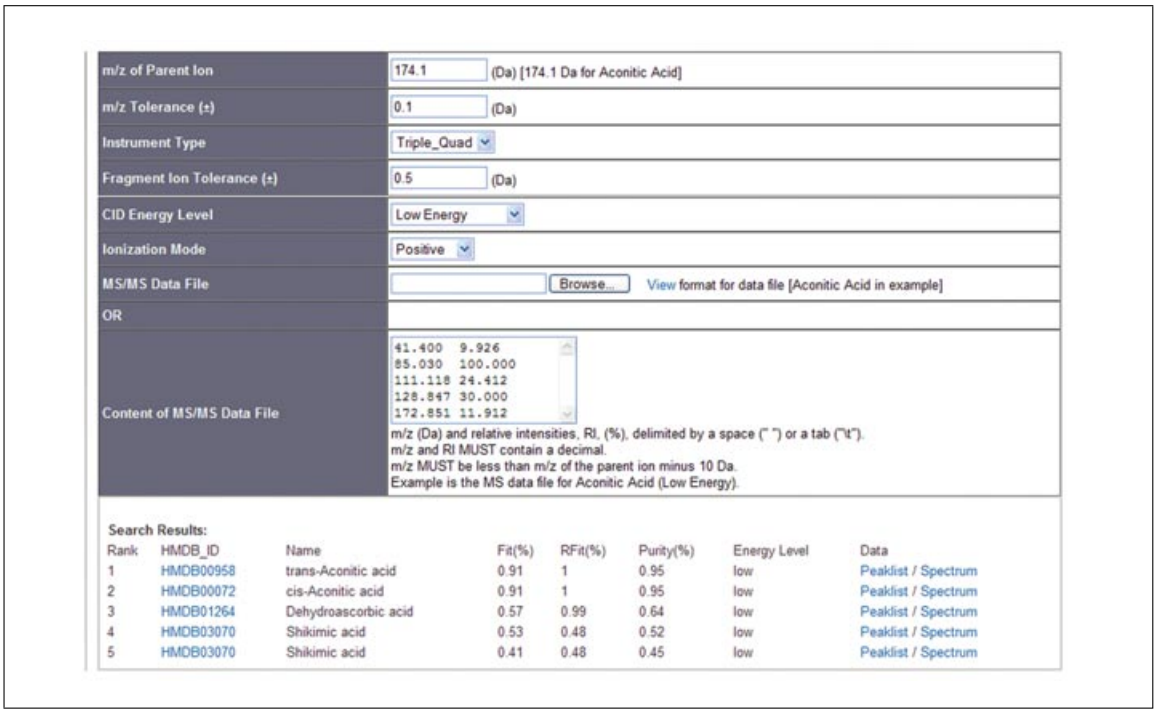

m/z of Parent Ion
174.1
(Da) [174.1 Da for Aconitic Acid]
m/z Tolerance (±)
0.1
(Da)
Instrument Type
Triple_Quad
Fragment Ion Tolerance (±)
0.5
(Da)
CID Energy Level
Low Energy
Ionization Mode
Positive
MS/MS Data File
Browse...
View format for data file [Aconitic Acid in example]
OR
Content of MS/MS Data File
41.400 9.926
85.030 100.000
111.118 24.412
128.867 30.000
172.851 11.912
m/z (Da) and relative intensities, RI, (%), delimited by a space (" ") or a tab ("\t").
m/z and RI MUST contain a decimal.
m/z MUST be less than m/z of the parent ion minus 10 Da.
Example is the MS data file for Aconitic Acid (Low Energy).
Search Results:
Rank HMDB_ID Name Fit(%) RFit(%) Purity(%) Energy Level Data
1 HMDB00958 trans-Aconitic acid 0.91 1 0.95 low Peaklist / Spectrum
2 HMDB00072 cis-Aconitic acid 0.91 1 0.95 low Peaklist / Spectrum
3 HMDB01264 Dehydroascorbic acid 0.57 0.99 0.64 low Peaklist / Spectrum
4 HMDB03070 Shikimic acid 0.53 0.48 0.52 low Peaklist / Spectrum
5 HMDB03070 Shikimic acid 0.41 0.48 0.45 low Peaklist / Spectrum

Figure 14.8.40 The MS/MS Search results appear at the bottom of the MS/MS Search page as an eight-column table.

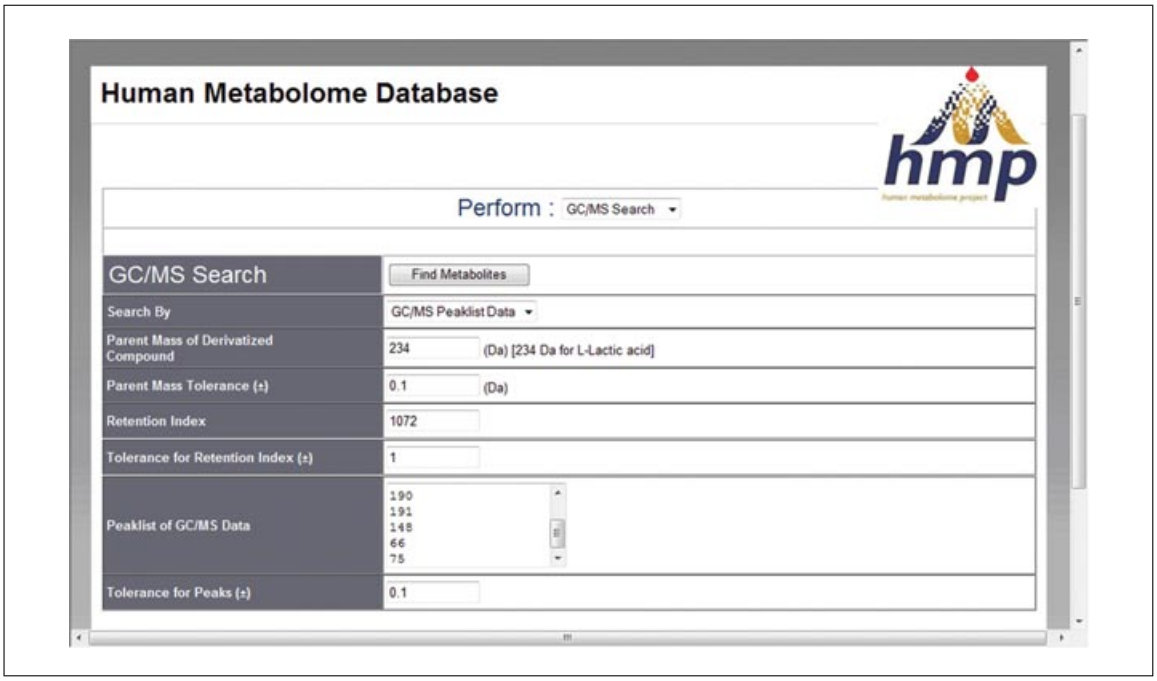


Figure 14.8.41 The filled out GC/MS Search page for L-lactic acid should appear as shown.

(**Parent Mass of Derivatized Compound**, **Parent Mass Tolerance**, **Retention Index**, **Tolerance for Retention Index**, **Peaklist of GC/MS Data**, and **Tolerance for Peaks**). The Parent Mass of Derivatized Compound represents the molecular weight of the compound of interest after chemical modification. For example, in the case of L-lactic acid with a monoisotopic mass of 90.03169 Da, the derivatized compound is bis(trimethylsilyl)lactate with a parent mass of 234 Da.

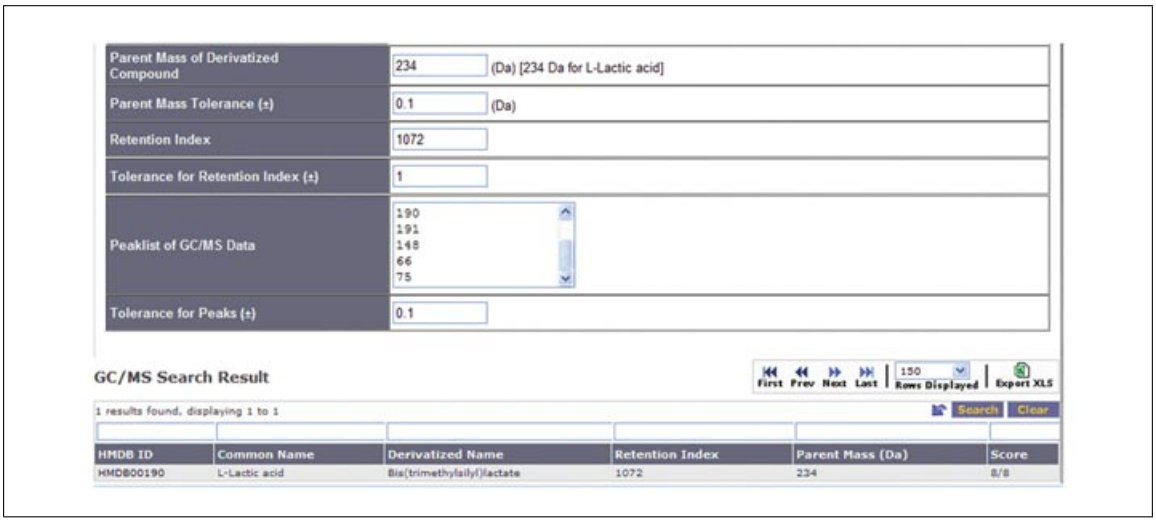


Figure 14.8.42 The GC/MS Results should appear in a multicolumn table below the query form.

15. In this example, we will look at L-lactic acid as the query compound. In the Parent Mass of Derivatized Compound field, enter `234`. For the other values, we will stick with the default values. If there are no values in the Peaklist of GC/MS Data field, enter the following values by typing each number and then clicking the Enter key, so that each value appears on a separate line: `73`, `147`, `117`, `190`, `191`, `148`, `66`, and `75`. The query page should appear as shown in Figure 14.8.41. Press the Find Metabolites button to launch the search.

16. After a few seconds, the results page should appear as shown in Figure 14.8.42. The results appear in a multi-column table with the following columns: **HMDB ID**, **Common Name**, **Derivatized Name**, **Retention Index**, **Parent Mass**, and **Score**. This table is easily sorted by clicking on the column header hyperlinks. The user can also search by any of the column fields by entering the query data into the text boxes that appear above each column name. In this case, with L-lactic acid as the query compound, there is only one hit (to itself). The HMDB column provides hyperlinked text which, if clicked, takes the user to the corresponding MetaboCard. The user is able to see the common name of the matching compounds as well as the name of the derivatized compound. The Score column on the far right indicates the number of query peaks that match the peak list for the compound in the database—in this case, 8 query peaks matched 8 peaks for L-lactic acid. As with the MS results tables, large hit lists can easily be navigated with the convenient arrow bars (**First**, **Previous**, **Next**, and **Last**) that appear above the results table. The user has the option of displaying 50, 100, or 150 rows at a time. The results table can also be exported as an Excel table by clicking on the **Export XLS** text or icon.

GUIDELINES FOR UNDERSTANDING RESULTS

Basic Protocol 1

This particular protocol was designed to show users how to explore the Human Metabolome Database (HMDB) and to learn about a given metabolite (1-methylhistidine), the enzymes that metabolize it, and the macromolecular partners that interact with it. The intent is to give users a broad overview of the data content and the capabilities of the HMDB. To summarize, steps 1 to 2 provide a brief description of the HMDB home page and its text search tool. Steps 3 to 13 take the user on a tour of a standard MetaboCard, highlighting the layout, content, and important visualization and display tools. Steps 14 to 19 describe how to use the HMDB Browser, Chemical Class Browser, Biofluid Browser, and Tissue Browser, while step 20 demonstrates how

the **TextQuery** tool can be used. Step 21 shows how the **Data Extractor** can be used to construct very specific and elaborate searches about certain metabolites or metabolic enzymes/macromolecular interacting partners, while step 22 highlights the content and information that can be obtained from the HMDB's Download page. Overall, the aim of this protocol is to provide sufficient grounding and rationale to allow users to more fully explore the HMDB on their own. It is also worth noting that this protocol did not cover every aspect of the HMDB's search and query capabilities. In particular, the **ChemQuery** and the spectral search (**NMR Search**, **MS/MS Search**, and **GC/MS Search**) options or their potential applications were not discussed. These query tools were discussed in separate protocols (Basic Protocols 2 and 3).

Basic Protocol 2

This protocol outlines the procedures for using the HMDB's chemical similarity search routines. Steps 3 to 5 describe how to use the HMDB's chemical structure drawing tools, specifically its ChemSketch Java applet. This series of steps illustrates how **ChemQuery** can be used find metabolites that are structurally related to other metabolites. Additionally steps 8 to 9 outline some of the other available chemical structure search utilities available in the HMDB, including the **Show Similar Structures** button that is available at the top of every MetaboCard. This feature allows users to identify other compounds in the HMDB that are structurally similar to their metabolite of interest. This kind of query is particularly useful for researchers wishing to do comparative metabolite analysis or comparative pathway analysis. It is worth noting that **ChemQuery**'s structure similarity search will not find all metabolites related to dopamine. This result demonstrates one of the algorithmic limitations of the **ChemQuery** search. Since the program only uses SMILES strings and SMILES substrings as part of its query process, it can sometimes miss structurally similar compounds. Furthermore, because SMILES strings depend on the atom ordering in the MOL file, the sequence in which one draws the query molecule in ChemSketch can change the syntax of the SMILES string. As a result, the scoring/ordering of compounds generated via a **ChemQuery** structure search of a known metabolite will differ from the scoring/ordering of compounds generated via a Show Similar Structures search. To address these problems, the **ChemQuery** search algorithm makes use of molecular weight, chemical formula, and identified chemical functionalities as part of its scoring scheme. More sophisticated structure search tools are available that use graph theory (subdirected graph isomorphisms) and structural superpositioning to identify similar compounds. PubChem, in particular, offers an excellent on-line structure query tool that employs these techniques.

Basic Protocol 3

This protocol outlines the procedures for identifying metabolites using the HMDB's spectral search routines. The intent is to give users a broad overview of the different types of spectral matching that are available to HMDB users. To summarize, steps 1 to 5 provide a concrete example of how to identify a single compound, in this case 1-methylhistidine, using NMR Search, while steps 6 to 7 demonstrate how to identify a mixture of compounds using NMR Search. Steps 8 to 10 outline the procedure for identifying compounds via MS Search. Steps 11 to 13 take the user through an example of how to perform an MS/MS search, while steps 14 to 16 illustrate how to search for metabolites using GC/MS Search. Overall, it is hoped that this protocol provides a sufficient sampling of the HMDB's spectral search capabilities, and that users will explore these capabilities further using query data that is relevant to their research.

One of the HMDB's great strengths is that it contains spectra of hundreds of common metabolites collected under controlled and clearly defined, aqueous conditions. There are many other spectral databases, for example, NMRShiftDB (Steinbeck et al., 2003), Spectral Database for Organic Compounds (SDBS; *http://riodb01.ibase.aist.go.jp/sdbs/*),

Golm Metabolome Database (Kopka et al., 2005), and the NIST Spectral Database (Ausloos et al., 1999), that contain spectra collected in organic solvents or spectra from compounds that are not metabolites, or, at least, not mammalian metabolites. It is useful to note that the HMDB has a comprehensive collection of NMR spectra (724 metabolites), MS/MS spectra (667 metabolites), and GC/MS spectra (231 metabolites), making it a valuable resource for the identification of metabolites by spectral matching. The usefulness of the HMDB's spectral search capabilities will only improve as the size of the spectral libraries increases.

COMMENTARY

Background Information

Enabling metabolomics research

Metabolomics is a relatively new addition to the "omics" sciences. As a consequence it is still evolving some of its basic computational infrastructure (Wishart, 2007). Whereas most data in the field of proteomics, genomics, or transcriptomics is readily available and easily analyzed through on-line electronic databases, most metabolomic data is still housed in books, journals, and other paper archives. Metabolomics also differs from the other "omics" sciences because of its strong emphasis on chemicals and analytical chemistry techniques such as NMR, GC-MS, or LC-MS. As a result, the analytical software used in metabolomics is often quite different than most of the software used in genomics, proteomics, or transcriptomics (Wishart, 2007). The field of metabolomics is not only concerned with the identification and quantification of metabolites, it is also concerned with relating metabolite data to genes, proteins, pathways, physiology, and phenotypes. As a result, metabolomics requires that whatever chemical information it generates must be linked to both biochemical causes and physiological consequences. This means that metabolomics must combine two very different fields of informatics: bioinformatics and cheminformatics.

Despite these differences, metabolomics still shares many of the same computational needs with genomics, proteomics, and transcriptomics. All four "omics" techniques require electronically accessible and searchable databases, all of them require software to interpret or process data from their own high-throughput instruments, and all require software tools to predict or model properties, pathways, and processes. These shared computational needs are the common thread that links metabolomics with all of the other "omics" sciences, and ultimately to systems biology.

A central focus of metabolomics is on characterizing dozens of metabolites at a time and then using these metabolites or combinations of metabolites to identify disease biomarkers or model large-scale metabolic processes. As a result, metabolomics researchers need databases that can be searched not just by pathways or compound names, but also by NMR spectra, MS spectra, GC-MS retention indices, chemical structures, or chemical concentrations. In addition to these query requirements, metabolomics researchers routinely need to search for metabolite properties, tissue/organ locations, or metabolite-disease associations. Therefore, metabolomics databases require information not only about compounds and reaction diagrams, but also data about physico-chemical properties, compound concentrations, biofluid or tissue locations, subcellular locations, known disease associations, nomenclature, descriptions, enzyme data, mutation data, and characteristic MS or NMR spectra. These data need to be readily available, experimentally validated, fully referenced, easily searched, and readily interpreted, and they need to cover as much of a given organism's metabolome as possible.

These are very tall orders, but the HMDB was constructed in an attempt to address all of the above-mentioned database needs. Indeed, a key feature that distinguishes the HMDB from other metabolic resources is its extensive support for higher-level database searching and selection functions. As seen in the tutorials provided here, the HMDB offers a wide variety of searching and browsing tools including a Boolean text search (Basic Protocol 1), a relational data extraction tool (Basic Protocol 1), a chemical structure search utility (Basic Protocol 2), a local BLAST search that supports both single and multiple sequence queries, an MS spectral matching tool (Basic Protocol 3), a GC-MS spectral matching facility (Basic Protocol 3), and an NMR spectral search tool (Basic Protocol 3). These spectral query tools are particularly useful for identifying compounds via MS or NMR data from other metabolomic studies.

One of the most obvious trends in computational metabolomics is the growing alignment or integration of metabolomics with systems biology. This integration will require that metabolomics methods and data reduction techniques will have to become much more quantitative. While chemometric methods for spectral analysis will likely continue to be popular among some groups for certain types of applications, the long-term trend in metabolomics seems to be toward rapid/high-throughput compound identification and quantification. These so-called targeted or quantitative methods will require greater reliance on spectral libraries and spectral standards and will, no doubt, lead to the appearance of organism-specific metabolite databases. This trend towards large-scale metabolite identification and quantification will likely encourage metabolomics researchers to adopt many of the analytical approaches commonly used in transcriptomics and proteomics, where transcript and protein levels are routinely quantified, compared, and analyzed. Given the importance that bioinformatics has played in establishing genomics and proteomics, it is likely that continuing developments in bioinformatics will have an equally profound impact on metabolomics and, ultimately, in its role in systems biology.

Critical Parameters and Troubleshooting

To facilitate consistency and simplicity, the HMDB has relatively few user-settable parameters. One component of the HMDB that can cause users some problems is the **Data Extractor** tool. This relational query system requires that the users know something about the content in different HMDB fields, including number ranges (for molecular weight or pK_a), or type of textual content. Obviously, typing in a negative molecular weight, a misspelled or nonsense word, a number where a word is expected, or a word where a number is expected will cause some unpredictable behavior in the search engine. If a questionable result is generated or if a query seems to "hang" for more than 1 min, users are requested to double-check the query to make sure it contains none of the above errors. Nonresponsiveness can be a problem with any Web site. This may reflect heavy use, periodic maintenance, server hardware problems, or the submission of an erroneously structured query that, in effect, searches and grabs all data in the database. The HMDB is heavily used and certainly its performance may be compromised by this heavy use (or abuse). If users experience consistent problems with either Web site access or program performance, they are encouraged to contact the HMDB staff or the authors of this unit.

The HMDB is a curated database, not an archival database. This means that the data in the HMDB are compiled, assessed, and entered by trained curators. Every effort is made to ensure the data in the HMDB is as correct, complete, and as current as possible.

However, as with any database, the HMDB contains some errors. These may be errors arising from data entry, metabolite deaccessioning (removing a metabolite but leaving the HMDB link in place), or recent revisions to our knowledge about a particular compound or associated enzyme. If any users believe they have identified an error, we would encourage them to contact the HMDB staff as soon as possible. Usually, errors can be confirmed and corrected within a few days. Likewise, users may find that some data are missing in certain MetaboCards. In many cases, the information (melting point, solubility, pK_a, enzyme, or transporter) has never been collected or is not yet known. However, if users become aware of a new source of information that fills in a missing data field, they are encouraged to contact the HMDB staff.

Acknowledgements

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Internet Resources

http://hmdb.ca/
Human Metabolome Database.

http://www.genome.jp/dbget-bin/www_bfind?compound
KEGG Ligand Database for Chemical Compounds.

http://biocyc.org/META/server.html
BioCyc.

http://bigg.ucsd.edu/
BiGG Database.

http://en.wikipedia.org/wiki/Main_Page
Wikipedia.

http://metlin.scripps.edu/metabo_search.php
Metlin.

http://pubchem.ncbi.nlm.nih.gov/
PubChem.

http://www.ebi.ac.uk/chebi/
ChEBI.

http://www.acdlabs.com/products/java/sda/
ACD/Structure Drawing Applet.

http://www.cmpharm.ucsf.edu/~walther/webmol.html
WebMol Web site.

http://www.rcsb.org/pdb/home/home.do
PDB.

http://www.bmrb.wisc.edu/
BMRB.

http://www.ncbi.nlm.nih.gov/pubmed/
PubMed.

http://www.ncbi.nlm.nih.gov/omim/
OMIM.

http://www.metagene.de/programm/tdb.prg?esp=index
Metagene.

http://www.genome.jp/dbget-bin/www_bfind?pathway
KEGG Pathway Database.

http://wishart.biology.ualberta.ca/SimCell/
SimCell.

http://www.ncbi.nlm.nih.gov/Genbank/
GenBank.

http://expasy.org/sprot/
Swiss-Prot.

http://www.geneontology.org/
Gene Ontology.

http://pfam.sanger.ac.uk/
Pfam.

http://www.genome.jp/dbget-bin/www_bfind?enzyme
KEGG Ligand Database for Enzyme Nomenclature.

http://www.genecards.org/index.shtml
GeneCards.

http://www.dsi.univ-paris5.fr/genatlas/
Genatlas.

http://www.genenames.org/
HUGO Gene Nomenclature Committee (HGNC).

http://www.ncbi.nlm.nih.gov/projects/SNP/
dbSNP.

http://nmrshiftdb.ice.mpg.de/
NMRShiftDB.

http://www.nist.gov/srd/nist1a.htm
NIST Spectral Database.

http://riodb01.ibase.aist.go.jp/sdbs/
Spectral Database for Organic Compounds.

http://csbdb.mpimp-golm.mpg.de/
Golm Metabolome Database.

ChEBI: An Open Bioinformatics and Cheminformatics Resource

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ABSTRACT

Chemical Entities of Biological Interest (ChEBI) is a freely available dictionary of molecular entities focused on "small" chemical compounds. This unit provides a detailed guide to browsing, searching, downloading, and programmatic access to the ChEBI database. *Curr. Protoc. Bioinform.* 26:14.9.1-14.9.20. © 2009 by John Wiley & Sons, Inc.

Keywords: chemical compound • chemical nomenclature • InChI • InChIKey • IUPAC • molecular entity • ontology • substructure search • similarity search • Web Services

INTRODUCTION

One cannot describe any entity or process in molecular biology without referring to molecular entities. Although bioinformatics came into existence primarily to serve the molecular biology community and traditionally was focused on biological macromolecules (proteins and nucleic acids), chemical entities are referred to frequently within biological databases. For instance, the molecules bound to a polypeptide chain are listed in the feature table of the UniProt database (*http://www.uniprot.org*); the names of substrates and products of enzymatic reactions populate the reaction field of IntEnz (*http://www.ebi.ac.uk/intenz/*); and drugs and mutagens affect the patterns of gene expressions and are reported as experimental conditions in microarray experiments deposited in ArrayExpress (*http://www.ebi.ac.uk/arrayexpress*; *UNIT 7.13*). However, since small chemical compounds are not the core data in these databases, they are typically present as free text in annotations. Free-text annotations are easy for a human audience to read and understand, but are difficult for computers to parse, can vary in quality from database to database, and can use different terminology to mean the same thing (even within the same database, if for example different annotators have used different terminology).

In addition to problems common to any free-text annotations, chemical entities pose a particularly difficult problem for annotation. Chemical names, especially common names, may contain ambiguity as to the exact structure of the molecular entity that is intended by the use of the name. For instance, the stereodescriptors are often dropped from the names of canonical amino acids; thus, the name "alanine" is frequently used instead of (presumably) L-alanine even though D-alanine is also synthesized in living organisms. On the other hand, there may be several valid names corresponding to the same compound. This derives from the use of different naming systems (Degtyarenko et al., 2007).

Chemical **E**ntities of **B**iological **I**nterest (ChEBI) is a freely available dictionary of molecular entities focused on such "small" chemical compounds. Molecules directly encoded by the genome (such as nucleic acids, proteins, and peptides derived from proteins by cleavage) are *not* as a rule included in ChEBI, as these are amply represented in other databases.

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ChEBI provides standardized descriptions of molecular entities that enable other databases at the EMBL-EBI and worldwide to annotate their entries in a consistent fashion. ChEBI focuses on high-quality manual annotation, nonredundancy, and provision of a chemical ontology rather than full coverage of the vast chemical space. In addition to molecular entities, ChEBI contains groups (parts of molecular entities) and classes of entities. A major feature of ChEBI is that it includes a chemical ontology, which allows the relationships between molecular entities or classes of entities and their parents and/or children to be specified in a structured way.

ChEBI uses nomenclature, symbolism, and terminology endorsed by the International Union of Pure and Applied Chemistry (IUPAC) and the Nomenclature Committee of the International Union of Biochemistry and Molecular Biology (NC-IUBMB). All the data in ChEBI is nonproprietary or derived from a nonproprietary source and is therefore freely available to anyone. In addition, each data item is fully traceable and explicitly referenced to the original source.

ChEBI aims to address the problems of chemical annotation within biological databases by providing a definitive reference controlled vocabulary and ontology of chemical entities which are of relevance to the biological community.

BASIC PROTOCOL 1

NAVIGATION AND SIMPLE SEARCH

ChEBI can be searched using a text query via a simple or advanced search. This protocol demonstrates how to perform a text search and familiarizes the user with the ChEBI Web pages.

Necessary Resources

Hardware

A computer with Internet access

Software

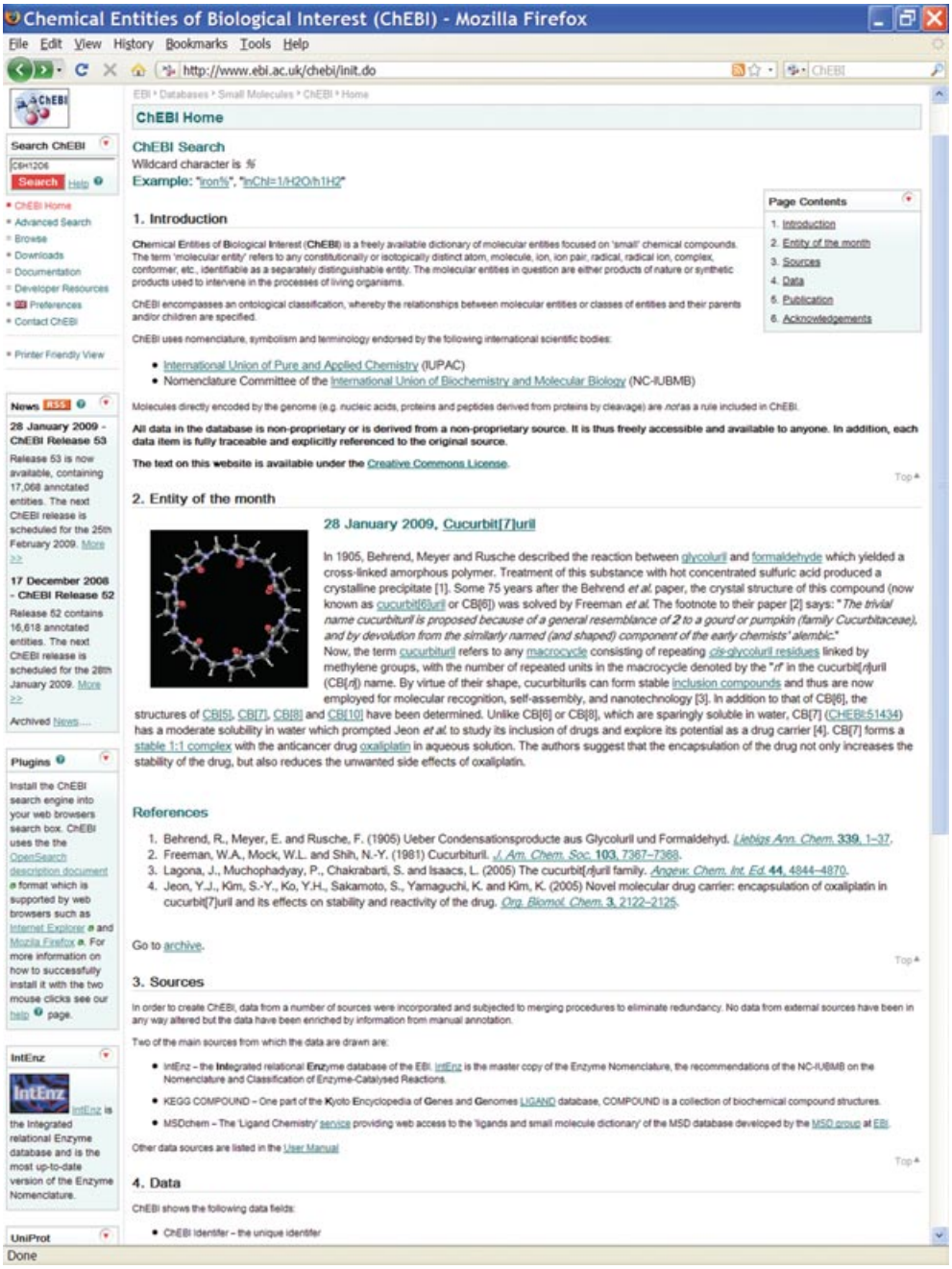
Internet browser, e.g., Internet Explorer (*http://www.microsoft.com/ie*), Netscape (*http://browser.netscape.com/*), Firefox (*http://www.mozilla.org/firefox/*), or Safari (*http://www.apple.com/safari*); Java version 5 or higher

Getting started

1. Use the Internet browser to open the ChEBI home page (*http://www.ebi.ac.uk/chebi/*; Fig. 14.9.1).

   This page is the starting point for exploring ChEBI. The body of the page contains an introduction to the scope of ChEBI, the featured Entity of the Month, a note on data sources, the list of data fields, the official ChEBI publication to cite, and Acknowledgements. The menu on the left of the main page facilitates the navigation through the ChEBI Web site. In the space under this menu, the latest news, updates, and developments are announced via an RSS Feed.

2. To change the user settings, click on Preferences in the left-hand side menu or use a link (*http://www.ebi.ac.uk/chebi/userSettingsForward.do*). Currently, the ChEBI Preferences menu allows the user to choose the language using a drop-down menu (from English, French, German, Russian and Spanish); the chemical structure view (static image or applet) using radio buttons; and the ChEBI Ontology view ("parents and children" only or tree view) using radio buttons. For this protocol, we recommend that Applet be chosen. After choosing the Preferences, click on the Submit Preferences button. The browser will remember the user settings for this and subsequent sessions.


Chemical Entities of Biological Interest (ChEBI) - Mozilla Firefox
File Edit View History Bookmarks Tools Help
http://www.ebi.ac.uk/chebi/init.do
ChEBI Home
ChEBI Search
Wildcard character is %
Example: "iron%", "InChI=1/H2O/h1H2"
Page Contents
1. Introduction
2. Entity of the month
3. Sources
4. Data
5. Publication
6. Acknowledgements
1. Introduction
Chemical Entities of Biological Interest (ChEBI) is a freely available dictionary of molecular entities focused on 'small' chemical compounds.
ChEBI encompasses an ontological classification, whereby the relationships between molecular entities or classes of entities and their parents and/or children are specified.
ChEBI uses nomenclature, symbolism and terminology endorsed by the following international scientific bodies:
International Union of Pure and Applied Chemistry (IUPAC)
Nomenclature Committee of the International Union of Biochemistry and Molecular Biology (NC-IUBMB)
Molecules directly encoded by the genome (e.g. nucleic acids, proteins and peptides derived from proteins by cleavage) are not as a rule included in ChEBI.
All data in the database is non-proprietary or is derived from a non-proprietary source. It is thus freely accessible and available to anyone. In addition, each data item is fully traceable and explicitly referenced to the original source.
The text on this website is available under the Creative Commons License.
2. Entity of the month
28 January 2009, Cucurbit[7]uril
In 1905, Behrend, Meyer and Rusche described the reaction between glycoluril and formaldehyde which yielded a cross-linked amorphous polymer. Treatment of this substance with hot concentrated sulfuric acid produced a crystalline precipitate [1]. Some 75 years after the Behrend et al. paper, the crystal structure of this compound (now known as cucurbit[6]uril or CB[6]) was solved by Freeman et al. The footnote to their paper [2] says: "The trivial name cucurbituril is proposed because of a general resemblance of 2 to a gourd or pumpkin (family Cucurbitaceae), and by devolution from the similarly named (and shaped) component of the early chemists' alembic."
Now, the term cucurbituril refers to any macrocycle consisting of repeating cis-glycoluril residues linked by methylene groups, with the number of repeated units in the macrocycle denoted by the "n" in the cucurbit[n]uril (CB[n]) name. By virtue of their shape, cucurbiturils can form stable inclusion compounds and thus are now employed for molecular recognition, self-assembly, and nanotechnology [3]. In addition to that of CB[6], the structures of CB[5], CB[7], CB[8] and CB[10] have been determined. Unlike CB[6] or CB[8], which are sparingly soluble in water, CB[7] (CHEBI:51434) has a moderate solubility in water which prompted Jeon et al. to study its inclusion of drugs and explore its potential as a drug carrier [4]. CB[7] forms a stable 1:1 complex with the anticancer drug oxaliplatin in aqueous solution. The authors suggest that the encapsulation of the drug not only increases the stability of the drug, but also reduces the unwanted side effects of oxaliplatin.
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2. Freeman, W.A., Mock, W.L. and Shih, N.-Y. (1981) Cucurbituril. J. Am. Chem. Soc. 103, 7367–7368.
3. Lagona, J., Muchophadyay, P., Chakrabarti, S. and Isaacs, L. (2005) The cucurbit[n]uril family. Angew. Chem. Int. Ed. 44, 4844–4870.
4. Jeon, Y.J., Kim, S.-Y., Ko, Y.H., Sakamoto, S., Yamaguchi, K. and Kim, K. (2005) Novel molecular drug carrier: encapsulation of oxaliplatin in cucurbit[7]uril and its effects on stability and reactivity of the drug. Org. Biomol. Chem. 3, 2122–2125.
Go to archive.
3. Sources
In order to create ChEBI, data from a number of sources were incorporated and subjected to merging procedures to eliminate redundancy. No data from external sources have been in any way altered but the data have been enriched by information from manual annotation.
Two of the main sources from which the data are drawn are:
IntEnz – the Integrated relational Enzyme database of the EBI. IntEnz is the master copy of the Enzyme Nomenclature, the recommendations of the NC-IUBMB on the Nomenclature and Classification of Enzyme-Catalysed Reactions.
KEGG COMPOUND – One part of the Kyoto Encyclopedia of Genes and Genomes LIGAND database, COMPOUND is a collection of biochemical compound structures.
MSDchem – The 'Ligand Chemistry' service providing web access to the 'ligands and small molecule dictionary' of the MSD database developed by the MSD group at EBI.
Other data sources are listed in the User Manual
4. Data
ChEBI shows the following data fields:
ChEBI Identifier – the unique identifier
Search ChEBI
Search
ChEBI Home
Advanced Search
Browse
Downloads
Documentation
Developer Resources
Preferences
Contact ChEBI
Printer Friendly View
News
28 January 2009 - ChEBI Release 53
Release 53 is now available, containing 17,068 annotated entities. The next ChEBI release is scheduled for the 25th February 2009. More >>
17 December 2008 - ChEBI Release 52
Release 52 contains 16,618 annotated entities. The next ChEBI release is scheduled for the 28th January 2009. More >>
Archived News ...
Plugins
Install the ChEBI search engine into your web browsers search box. ChEBI uses the the OpenSearch description document format which is supported by web browsers such as Internet Explorer and Mozilla Firefox. For more information on how to successfully install it with the two mouse clicks see our help page.
IntEnz
IntEnz is the Integrated relational Enzyme database and is the most up-to-date version of the Enzyme Nomenclature.
UniProt
Done

Figure 14.9.1 The ChEBI home page.

3. To use the simple text search, just enter (type or copy) your search query into the search box located at the top left of the ChEBI front page. The search query may be any data associated with an entity such as a name, formula, CAS Registry Number, InChI, or InChIKey string (Heller and McNaught, 2009). For instance, type the formula `C6H12O6` into the search box.

4. Click on the Search button and view the list of search results (entitled ChEBI Results). When there are multiple results returned from a search, the search takes you to a search results page. Search results may be exported for import into other applications. When there is only one result, the search takes you directly to the entity result page, bypassing the search results table.

Wildcards are available for both the simple and the advanced search. The wildcard character is "%". A wildcard character allows you to find compounds by typing in a partial name. The search engine will then try to find names matching the pattern you have specified using the wildcard character. To match terms that start with your search term, add the wildcard character to the end of your query. For example, searching for `aceto%` *will find compounds such as acetochlor, acetophenazine, and acetophenazine maleate. To match terms that end with your search term, add the wildcard character to the start of your query. For example, searching for* `%azine` *will find compounds such as 2-(pentaprenyloxy)dihydrophenazine, acetophenazine, and 4-(ethylamino)-2-hydroxy-6-(isopropylamino)-1,3,5-triazine. Any number of wildcard characters may be used within a search term, thus affording considerable scope within the search facility. To match terms that contain your search term, add the wildcard character to the start and the end of your query. For example, searching for* `%propyl%` *will find compounds such as (R)-2-hydroxypropyl-CoM, 2-isopropylmaleic acid, and 2-methyl-1-hydroxypropyl-TPP.*

View a ChEBI entry page

5. On the ChEBI Results page, click on any of the ChEBI identifier hyperlinks to navigate to the individual ChEBI entry. A sample ChEBI entry (Main page) is shown in Figure 14.9.2.

   The main page of a typical ChEBI entry may contain: (1) a unique, unambiguous, recommended ChEBI name (e.g., cisplatin) and an associated stable unique identifier (e.g., CHEBI:27899), (2) molecular formula (e.g., H6Cl2N2Pt), (3) a diagram of the chemical structure, where appropriate (particular compounds and groups, but generally not classes), as well as data derived from chemical structures, such as InChI, InChIKey and SMILES strings, charge and mass, (4) a definition where appropriate (mostly for classes), (5) a collection of synonyms, including the IUPAC recommended name for the entity where appropriate, and brand names and INNs for drugs. Flags are used to indicate different languages, where synonyms are not in English, (6) a collection of cross-references to other databases (where these are sourced from non-proprietary origins) and Registry Numbers, and (7) links to the ChEBI ontology.

6. To see the automatic cross-reference page, click on the Automatic Xrefs tab at the top of the main entry view screen (see Fig. 14.9.3).

   A number of databases are automatically cross-referenced to ChEBI entities for each release, and these automatic cross-references are found in the Automatic Xrefs page. This page contains the "EB-eye"-style (http://www.ebi.ac.uk/inc/help/search_help.html) classification of the databases by categories.

7. The chemical structure of the entry is shown in the upper-left corner of the main entry page. Some ChEBI entries contain more than one representation of the same chemical structure. Use entry CHEBI:48095 as an example. To see all structures, click on "more structures >>." To manipulate the structures, double-click on a structure of interest. This will invoke the MarvinView applet in a separate window (Fig. 14.9.4). The MarvinView visualization is especially useful for 3-D structures.

8. To save a structure of interest in MDL .mol format, click on the Molfile link next to the diskette icon. For instance, find corrin (CHEBI:33221) and save the default structure. ChEBI automatically assigns file name `ChEBI_33221.mol`.

9. To print out the ChEBI entry, use the Printer Friendly View option on the ChEBI menu.

Navigating the ChEBI ontology

10. The ChEBI ontology can be used to navigate to related entries within ChEBI via the ChEBI ontology section within an entry page. To determine whether an entity is an instance of another entity, look for the "**is a**" relationship in the ChEBI Ontology under the Parents sub-heading. For example, find chloroform (CHEBI:35255) and you can determine that chloroform is a chloromethane (CHEBI:23148) by the use of the **is a** relationship in the ChEBI Ontology section. Click on chloromethanes and

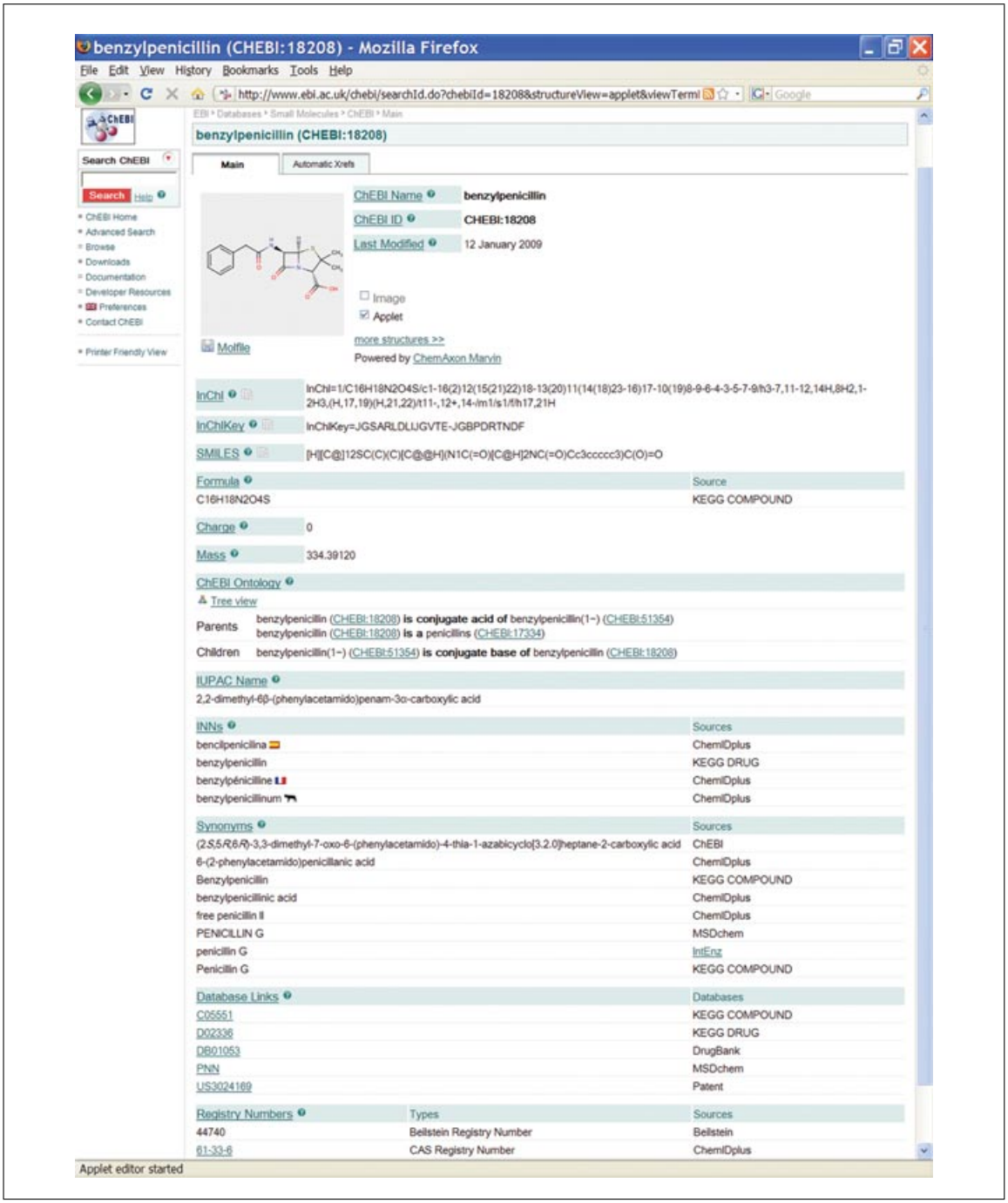

Figure 14.9.2 The main page of a sample ChEBI entry.

you see that "chloromethanes" is itself an instance of chloroalkanes (CHEBI:23128). You can continue navigating the ontology by clicking on the **is a** relationships in the Parents section.

Is a *implies that Entity A is an instance of Entity B.*

11. You can determine parthood within the ChEBI ontology by using the **"has part"** relationship. For example, find the entry tetracyanonickelate(2–) (CHEBI:49928) and scroll down to the ChEBI Ontology section. Click on potassium tetracyanonickelate(2–) (CHEBI:30071), which you can see has part tetracyanonickelate(2–) (CHEBI:49928).

Has part *is used to indicate the relationship between the whole and a part of it.*

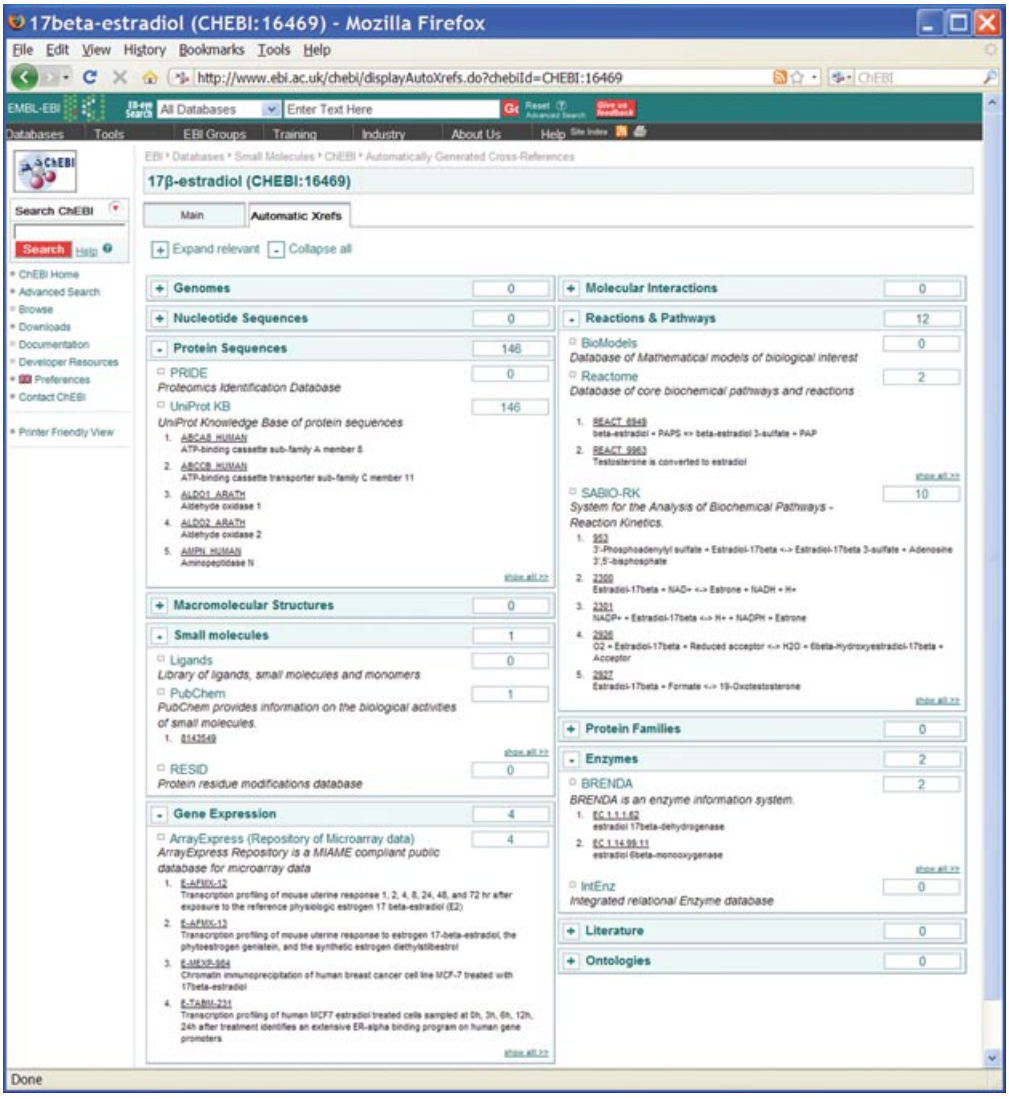

Figure 14.9.3 The Automatic Xrefs page of a sample ChEBI entry.

12. By using the "**is conjugate acid of**" and "**is conjugate base of**" relationships, you can find entries that are related via their conjugate acids or their conjugate bases. For example, find the neutral pyruvic acid (CHEBI:32816) and scroll down to the ChEBI Ontology section. You can determine that pyruvic acid is the conjugate acid of the pyruvate anion (CHEBI:15361), while as a corollary pyruvate is the conjugate base of the acid.

    Is conjugate base of *and* ***is conjugate acid of*** *are cyclic relationships used to connect acids with their conjugate bases.*

13. The "**is tautomer of**" relationship allows you to find related compounds interconnected via the chemical reaction called tautomerization. Find the entry L-serine (CHEBI:17115) and scroll down to the ChEBI Ontology section to view its tautomer zwitterion (CHEBI:33384), indicated by the **is tautomer of** relationship.

    Is tautomer of *is a cyclic relationship used to show the interrelationship between two tautomers, where the differences between the structures are significant enough to warrant their separate inclusion in ChEBI. Tautomers are defined as isomers that differ only in the positions of hydrogen atoms and electrons, the remainder of the skeletons being the same.*

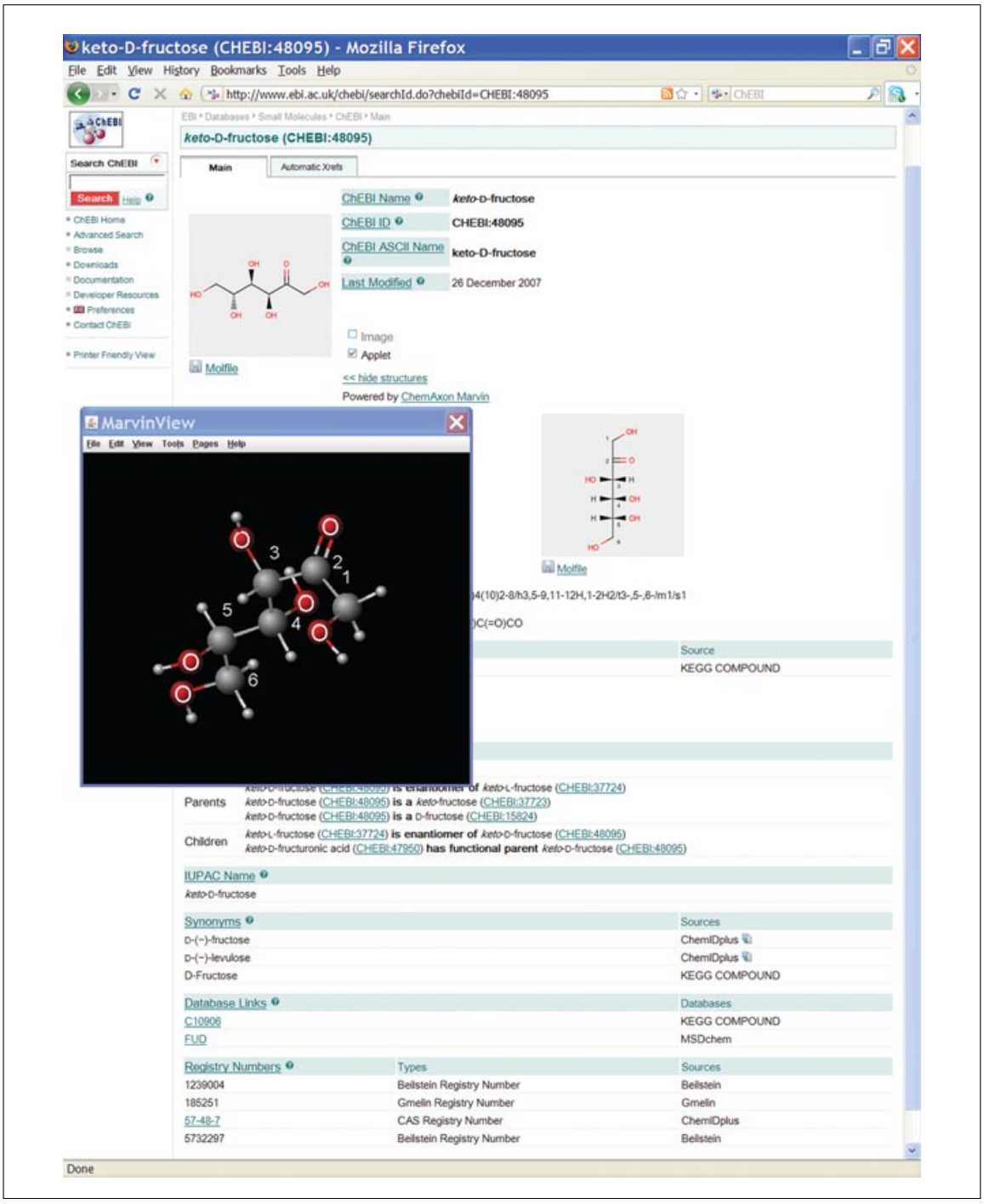

Figure 14.9.4 The MarvinView applet.

14. You can use the "**is enantiomer of**" relationship to find mirror images of a chemical structure. Find the entry D-alanine (CHEBI:15570) and scroll down to the ChEBI Ontology section. Click on L-alanine (CHEBI:16977), which is related via the **is enantiomer of** relationship and note that the chemical structures are mirror images of each other.

    Is enantiomer of *is a cyclic relationship used when two entities are mirror images of and nonsuperposable upon each other.*

15. The "**has functional parent**" relationship allows you to find related molecular entities based on whether an entity has one or more characteristic groups from which the other

can be derived from functional modification. Find the entry 16α-hydroxyprogesterone (CHEBI:15826) and scroll down to the ChEBI Ontology section. Here you can see that 16α-hydroxyprogesterone can be derived by functional modification (in this case, 16α-hydroxylation) of progesterone (CHEBI:17026).

Has functional parent *is used to denote the relationship between two molecular entities (or classes of entities), one of which possesses one or more characteristic groups from which the other can be derived by functional modification.*

16. Find the entry 1,4-naphthoquinone (CHEBI:27418) and scroll down to the ChEBI Ontology section. Here you can see that 1,4-naphthoquinone (CHEBI:27418), via the "**has parent hydride**" relationship, has as its parent hydride the cyclic hydrocarbon naphthalene (CHEBI:16482).

    Has parent hydride *denotes the relationship between an entity and its parent hydride. The parent hydride is defined by IUPAC as "an unbranched acyclic or cyclic structure or an acyclic/cyclic structure having a semisystematic or trivial name to which only hydrogen atoms are attached" (http://goldbook.iupac.org/P04405.html).*

17. You can find substituent groups related to an entity by using the "**is substituent group from**" relationship. Find the entity L-valino group (CHEBI:32854) and scroll down to the ChEBI Ontology section of the entry. Here you can see that the L-valino group (CHEBI:32854) is derived by a proton loss from the N atom of L-valine (CHEBI:16414).

    Is substituent group from *indicates the relationship between a substituent group (or atom) and its parent molecular entity, from which it is formed by loss of one or more protons or simple groups such as hydroxy groups.*

18. The "**has role**" relationship allows you to see the particular behavior that the entity of interest may exhibit. Find the entry pseudoephedrine (CHEBI:51209) and scroll down to the ChEBI Ontology section. Here you can see that pseudoephedrine has roles of sympathomimetic agent (CHEBI:35524), bronchodilator agent (CHEBI:35523), and anti-asthmatic drug (CHEBI:49167).

    Has role *is used to denote the relationship between a molecular entity or a subatomic particle and a role it may play, either naturally or by means of human application. This is the only relationship that is allowed between the Molecular Structure and Role ontologies, or between the Subatomic Particle and Role ontologies.*

Browse ChEBI via the Periodic Table

19. Click on the left-hand menu item labeled Browse to expand the browse menu, and then click again on the Periodic Table link to open up the periodic table browser interface. Click on the symbol for Oxygen to browse the classes of molecular entities containing Oxygen.

20. The Periodic Table browser differentiates between molecular entities and the elements. Click on the header tab Elements to open the Periodic Table browser for the elements. Clicking on Oxygen now takes you to the entry page for the Oxygen element.

Browse ChEBI via the ontology

21. Click on the Browse Ontology menu in the left-hand corner of the page. This link will take you to the Ontology Lookup service (Côté et al., 2006; *http://www.ebi.ac.uk/ontology-lookup/*), which will allow you to browse the data in ChEBI via its three sub-ontologies, namely molecular structure, role, and subatomic particle.

    ChEBI Ontology is subdivided into three separate sub-ontologies: (1) Molecular Structure, in which molecular entities or parts thereof are classified according to composition and structure, e.g., hydrocarbons, carboxylic acids, tertiary amines; (2) Role, which classifies

entities either on the basis of their role within a biological and chemical context (e.g., antibiotic, antiviral agent, coenzyme, hormone, acid, base) or on the basis of their intended use by humans (e.g., pesticide, antirheumatic drug, fuel); and (3) Subatomic Particle, which classifies particles which are smaller than atoms, e.g., electron, photon, nucleon.

22. Expand the molecular structure sub-ontology by clicking on the + (plus) icon to the left of the term. You will see that molecular structure is sub-divided into two classes, namely molecular entities and groups. You can navigate further by similarly expanding any child terms within the ontology.

23. Expand the following path: "molecular entities," "inorganic molecular entities," "inorganic salt," "inorganic chloride salt," and then click on the child term "zinc dichloride." On the right-hand side of the screen, a number of synonyms will appear relating to this term (zinc dichloride). Scroll down the browser page to the Term Hierarchy graphical display, which is on the right-hand side of the screen, below the synonyms and cross-references box. The diagram shows all paths to the ChEBI ontology root from the selected term (zinc dichloride, in this example).

ADVANCED SEARCH

BASIC PROTOCOL 2

The advanced search provides for additional granularity of category to search in, as well as the option of using the Boolean operations when searching. The structure (substructure and similarity) search allows a chemical diagram to be used as a search query. Both text and structure search can be combined within a single query. This protocol demonstrates how to perform both an advanced text search and a structure search.

Necessary Resources

Hardware

A computer with Internet access

Software

Internet browser, e.g., Internet Explorer (*http://www.microsoft.com/ie*), Netscape (*http://browser.netscape.com/*), Firefox (*http://www.mozilla.org/firefox/*), or Safari (*http://www.apple.com/safari*); Java version 5 or higher

Structure-based search

The structure-based search facility within ChEBI allows you to search for structures in the database based on a provided structure, which may be drawn or uploaded.

1. Use the Internet browser to open the ChEBI home page (*http://www.ebi.ac.uk/chebi/*; Fig. 14.9.1).

2. To access the advanced search, select the Advanced Search link from the left-hand menu. This will open a screen showing the search box shown in Figure 14.9.5.

3. Use the ChemAxon MarvinSketch applet to enter structures.

   The top menu bar allows access to most of the available functionality of the sketching applet, grouped into menus for file manipulation, general editing functionality such as copy/paste, viewing manipulations such as display and color options, and various structure-drawing utilities. In addition to the top menu bar, various structure-drawing tools and utilities are available from the left-hand graphical button menu bar, various atom options from the right-hand-side button menu bar, and structure templates from the bottom-button menu bar. To upload the structure in one of the chemical formats, such as .mol or .pdb, go to File→ Open and navigate through your file system to choose the file. A comprehensive collection of animations illustrating the use of MarvinSketch, including structure drawing and file operations, is available at the ChemAxon Web site (http://www.chemaxon.com/anim/marvin/sketch_bond/drawbond.html).

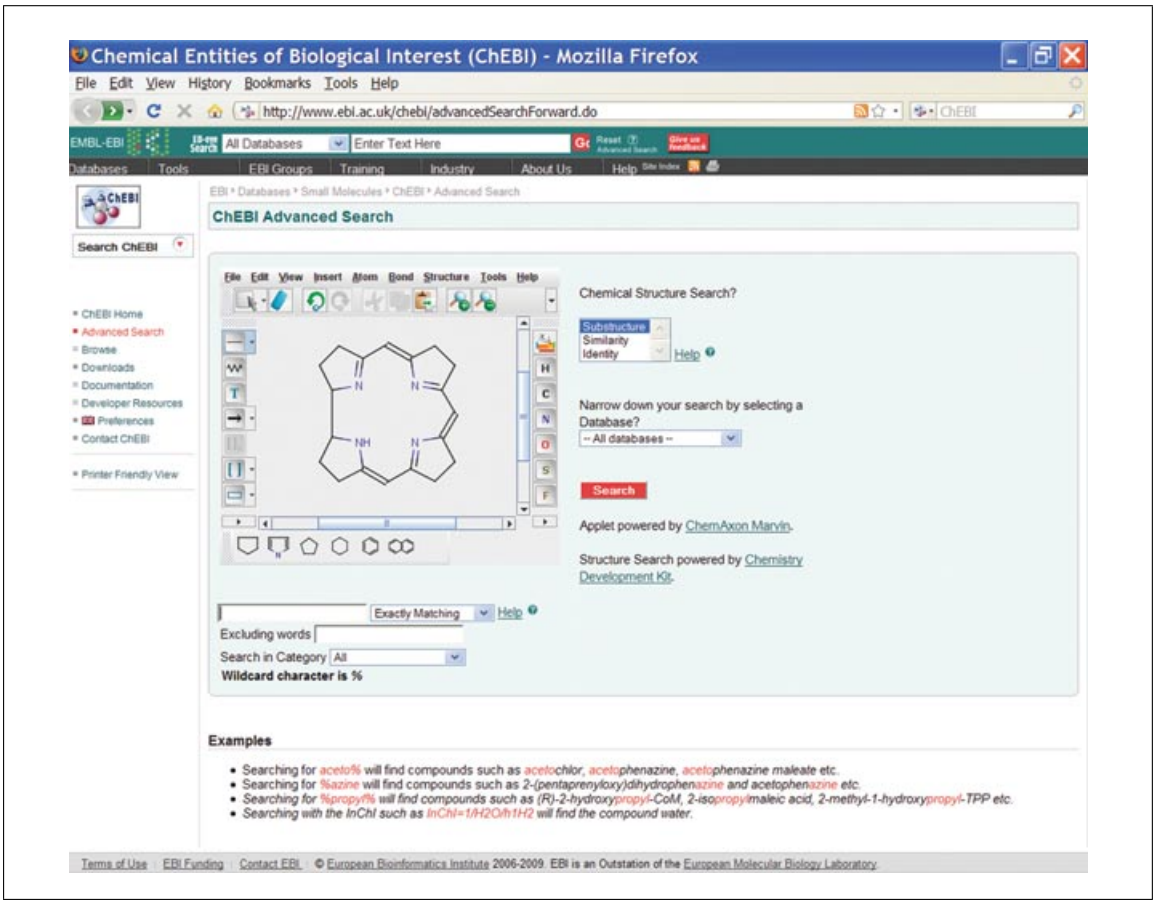

Figure 14.9.5 The Advanced Search page.

4. After the structure is drawn or uploaded, choose one of three available search options: Substructure, Similarity, or Identity.

5. For example, upload the structure of corrin (ChEBI_33221.mol) saved in Basic Protocol 1, choose Identity, and click on the Search button. As expected, the search brings just one result, viz. CHEBI:33221.

   Identity searches are based on the InChI, which means that an InChI is generated from the drawn or uploaded structure, and the database is then searched for exact matches to that InChI. This means that the identity search is subject to the same limitations as the uniqueness of InChIs. For example, searching for structures identical with cisplatin (CHEBI:27899) returns both cisplatin and transplatin (CHEBI:35852), since these have identical InChIs. However, in most cases an identity search will take you directly to the entry page for the single structure you have drawn, if it exists in the database.

6. You can reuse your structural query simply by hovering with the pointer over the structure diagram. This time, choose the Substructure search and left-click.

   Chemical substructure and similarity searches within ChEBI are based on the Chemistry Development Kit (CDK) fingerprints (Steinbeck et al., 2006; http://cdk.sourceforge.net). A fingerprint of a chemical structure is a way of representing special characteristics of that structure in an easily searchable form. For substructure searching, fingerprints are used as effective screening devices to narrow the set of candidates for a full substructure search. If all bits in a query fingerprint are also present in the target fingerprint of a stored database structure, this structure is subjected to the computationally expensive subgraph-matching algorithm. These bit operations are very fast and independent of the number of atoms in a structure due to the fixed length of the fingerprint.

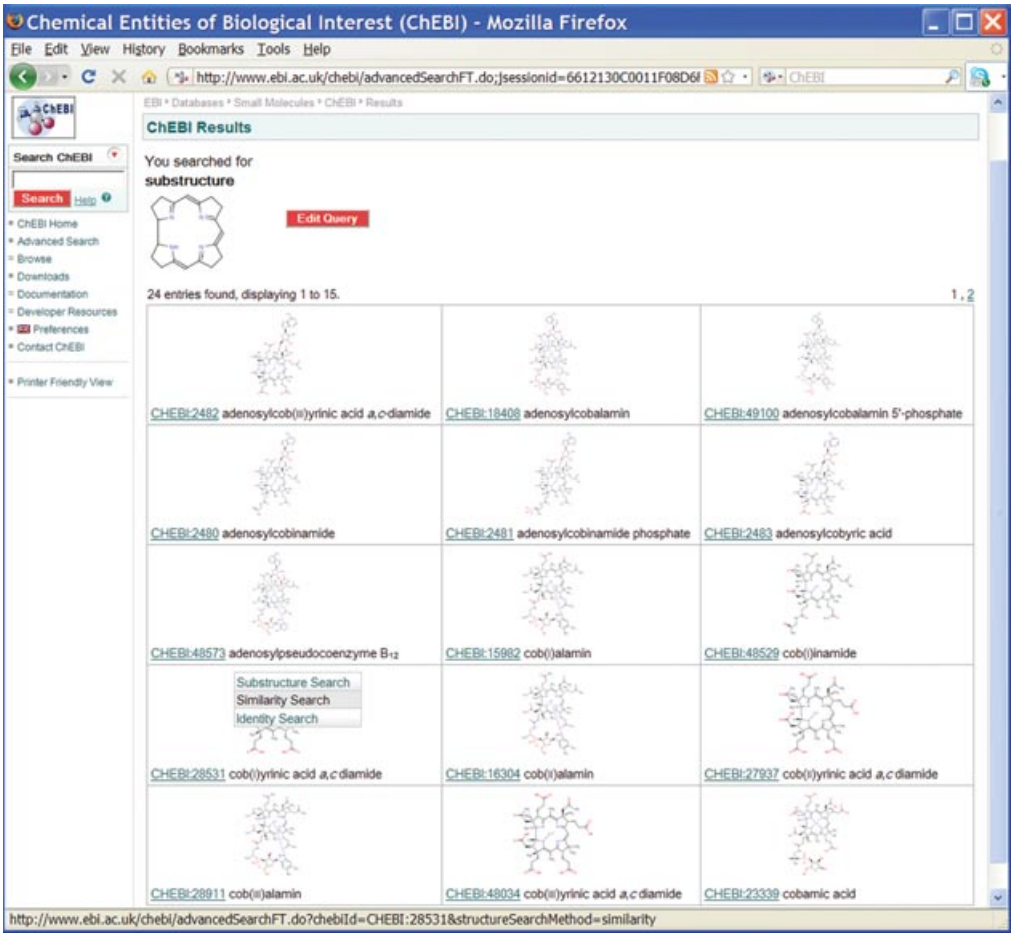

Figure 14.9.6 An Advanced Search result page.

7. On the Result page (Fig. 14.9.6), clicking on the relevant ChEBI accession hyperlinked under the search result image takes you to the entry page for that entity. In addition, further structure-based searches may be performed by hovering the pointer over any of the displayed images in the Results page (including the image of the original structure query) and clicking on one of the search options, which passes that structure directly to the search facility. For example, place the pointer over the image of the original structure query and choose the Similarity search.

   For similarity searching in ChEBI, fingerprints are used as input to the calculation of the similarity of two molecules in the form of the Tanimoto coefficient, which is calculated as the ratio:

$$T(a,b) = c/(a + b - c)$$

   where c is the count of bits "on" (i.e., 1 not 0) in the same position in both of the two fingerprints, a is the count of bits on in object A, and b is the count of bits on in object B (Kochev et al., 2003). The Tanimoto coefficient varies in the range 0.0 to 1.0, with a score of 1.0 indicating that the two structures are very similar (i.e., their fingerprints are the same).

Text-based search

The text-based search facility in the Advanced Search allows you to combine your search terms in order to narrow down your results. In addition, you can combine search terms with the structure-based search.

8. The operator "All of these words" (AND) allows you to find a compound that contains all of your search terms. For example, if you are searching for an organic acid with formula `C6H12O6`, specifying `acid C6H12O6` as the search term and selecting All of these words as the search option will retrieve several acids including fuconic acid and rhamnonic acid.

   ChEBI provides the standard Boolean operators when searching for compounds. All search terms need to be separated by a blank space.

9. The operator "Any of these words" (OR) allows you to type two or more words. It then tries to find a compound that contains at least one of these words. For example, if you wanted to find all compounds that contain either "silver" or "argent" as part of their names or synonyms, type in the search string `%silver% %argent%`.

10. Sometimes, common words can be a problem when searching, as they can provide too many results. The "Excluding words" (NOT) option can be used to limit the result set. For example, if you were looking for a compound related to chlorine but excluding acidic compounds, you could specify `%chlor%` as your search string but qualify the search by specifying `acid` in the excluding words field.

    The results of an advanced search are displayed in a grid. The results are paginated if >15 results are retrieved, and if only one result is returned then the entry page for that entity is loaded directly.

Searching in categories

This option allows you to narrow down your search by using the categories provided. The categories include:

All: this allows you to search all the categories.
ChEBI ID: allows searching for specific ChEBI identifiers.
ChEBI Name: will search only for ChEBI names matching your search term.
Synonym: will search in all the synonyms available for this compound.
IUPAC Name: will search for IUPAC names.
Database Accession: allows searching for accession numbers from other sources.
Formula: will search for formula.
Registry Number: will search for CAS, Beilstein, or Gmelin Registry Numbers matching your search criteria.
InChI/InChIKey: will search for InChI/InChIKey(s) matching your search criteria.
SMILES: will search for SMILES matching your search criteria.
Comment: allows searching for comments provided by the ChEBI annotators.

11. For example, type `007` and choose category Database Accession.

Searching with structures and text

This option allows you to narrow down your result set by combining your structure-based search and your text-based search as a Boolean AND operation.

12. For example, go to the entry 1*H*-pyrrole (CHEBI:19203). Copy the chemical structure into your clipboard by double clicking on the applet and selecting the Edit menu with the option to Copy. Navigate to the Advanced Search page and paste the chemical structure into the applet using the Edit menu on the applet. Type in `%N2%` and select the category Formula to narrow down your search to include two nitrogen atoms. Only substructures that have two nitrogen atoms in their formula will be returned as part of the result set.

13. You may narrow down your search by selecting a cross-referenced database (default is "All databases"). Use the structure of 1*H*-pyrrole to make a substructure search and choose Beilstein database. The word Beilstein appears over the selection menu. Use

the same menu to choose ChemIDplus database. The word ChemIDplus appears over the selection menu. The default Boolean operation is AND, therefore only the entries that have 1*H*-pyrrole as a substructure and have both Beilstein Registry Numbers and cross-references to ChemIDPlus will be returned as part of the result set. (If you choose the Boolean operation OR, only the entries which have 1*H*-pyrrole as a substructure and have either Beilstein Registry Numbers or cross-references to ChemIDPlus will be returned as part of the result set.)

ACCESSING ChEBI VIA WEB SERVICES

BASIC PROTOCOL 3

The ChEBI Web Service provides a means for programmatic access to the ChEBI dataset. This allows users to create their own applications, which query the ChEBI dataset from within their application, without having to download and locally incorporate the full dataset every time there is a new release. Web services are implemented as server applications, to which many clients may connect over the Internet.

Necessary Resources

Hardware

A computer with Internet access

Software

Internet browser, e.g., Internet Explorer (*http://www.microsoft.com/ie*), Netscape (*http://browser.netscape.com/*), Firefox (*http://www.mozilla.org/firefox/*), or Safari (*http://www.apple.com/safari*); Java version 5 or higher
Suitable programming language editor such as Eclipse

1. Go to the ChEBI Web Services page, which may be accessed from the menu on the left-hand side of the main page or directly via a link (*http://www.ebi.ac.uk/chebi/webServices.do*). This page describes the ChEBI Web service implementation and allows you to test the methods available on the Web service and examine the output.

   *There are four methods provided with which to access data. They are **getLiteEntity**, which retrieves a LiteEntityList and takes as parameters a search string and a search category (which may be null to search across all categories); **getCompleteEntity**, which retrieves the full data for an entity including synonyms, database links and structures, and which takes as parameter the ChEBI identifier; **getOntologyParents**, which retrieves the parents of the given entity (specified by ChEBI identifier) in the ChEBI ontology; and **getOntologyChildren**, which retrieves the children of the given entity (specified by ChEBI identifier) in the ChEBI ontology.*

```
package test.webapps;

import uk.ac.ebi.chebi.webapps.chebiWS.client.ChebiWebServiceClient;

public class TestChebiWebService {

    public static void main(String[] args) {
            ChebiWebServiceClient client = new ChebiWebServiceClient();
            //client provides entry to web service methods

        }
}
```

Figure 14.9.7 Java code illustrating the construction of the ChEBI Web Service client.

```
package test.webapps;

import uk.ac.ebi.chebi.webapps.chebiWS.client.ChebiWebServiceClient;
import uk.ac.ebi.chebi.webapps.chebiWS.model.ChebiWebServiceFault_Exception;
import uk.ac.ebi.chebi.webapps.chebiWS.model.LiteEntity;
import uk.ac.ebi.chebi.webapps.chebiWS.model.LiteEntityList;
import uk.ac.ebi.chebi.webapps.chebiWS.model.SearchCategory;

public class TestChebiWebService {

        /**
         * @param args
         */

        public static void main(String[] args) {
                ChebiWebServiceClient client = new ChebiWebServiceClient();

                try {
                        LiteEntityList benzenes =
                                client.getLiteEntity("benzene", SearchCategory.ALL);

                        for (LiteEntity le : benzenes.getListElement() ) {
                                System.out.println( le.getChebiAsciiName()
                                                + ", " + le.getChebiId() );

                        }

                } catch (ChebiWebServiceFault_Exception e) {
                        e.printStackTrace();
                }


        }

}
```

Figure 14.9.8 Java code illustrating the Web Service search capabilities.

*Thus, the search entry point into the dataset is the **getLiteEntity** method, which allows you to specify a search string and use it to search the database across one or all categories. Allowed search categories are (available as the SearchCategory enumeration in the domain model) listed above step 11 of Basic Protocol 2.*

*The search method returns a LiteEntityList, which may contain many LiteEntitys. For each LiteEntity contained in the list, the ChEBI ID may then be used to retrieve the full dataset by passing it as a parameter to the **getCompleteEntity** method. The Entity object, which is then returned contains the full ChEBI dataset linked to that identifier, including structures, database links and registry numbers, formulae, names, synonyms, and parent and children ontology relationships.*

*Navigating the ontology, without retrieving a complete Entity for each data item in the ontology, is accomplished by using the methods **getOntologyParents** (for navigating up towards the root) and **getOntologyChildren** (for navigating downwards towards the leaves). Note that an OntologyDataItem represents a relationship, specifying the ChEBI ID of the related term and the type of the relationship. The ChEBI ID can then be used to access the complete Entity if required. Some relationship types are cyclic, in which case the flag cyclicRelationship will be set to true. Cyclic relationships should be ignored for purposes of navigation, or if navigating them is required, care should be taken to traverse them only once.*

2. Download the client (Java or Perl) or generate your own client based on the ChEBI WSDL (*http://www.ebi.ac.uk/webservices/chebi/webservice?wsdl*). Place the client in the class path for your custom Web service client application. An example Java application is shown in Figure 14.9.7.

```
package test.webapps;

import uk.ac.ebi.chebi.webapps.chebiWS.client.ChebiWebServiceClient;
import uk.ac.ebi.chebi.webapps.chebiWS.model.ChebiWebServiceFault_Exception;
import uk.ac.ebi.chebi.webapps.chebiWS.model.DataItem;
import uk.ac.ebi.chebi.webapps.chebiWS.model.Entity;

public class TestChebiWebService {

        /**
         * @param args
         */

        public static void main(String[] args) {
                ChebiWebServiceClient client = new ChebiWebServiceClient();

                try {

                        Entity benzene = client.getCompleteEntity("CHEBI:16716");

                        for (DataItem link : benzene.getDatabaseLinks()) {
                                System.out.println(link.getData());

                        }

                } catch (ChebiWebServiceFault_Exception e) {
                        e.printStackTrace();

                }


        }
```

Figure 14.9.9 Java code illustrating the full retrieval of a ChEBI entry.

```
package test.webapps;

import uk.ac.ebi.chebi.webapps.chebiWS.client.ChebiWebServiceClient;
import uk.ac.ebi.chebi.webapps.chebiWS.model.ChebiWebServiceFault_Exception;
import uk.ac.ebi.chebi.webapps.chebiWS.model.OntologyDataItem;
import uk.ac.ebi.chebi.webapps.chebiWS.model.OntologyDataItemList;

public class TestChebiWebService {

        /**
         * @param args
         */
        public static void main(String[] args) {
                ChebiWebServiceClient client = new ChebiWebServiceClient();

                try {
                        traverseOntologyParents(client, "CHEBI:16716");

                } catch (ChebiWebServiceFault_Exception e) {
                        e.printStackTrace();
                }

        }

        private static void traverseOntologyParents(ChebiWebServiceClient client,
        String chebiId) throws ChebiWebServiceFault_Exception {

                OntologyDataItemList parents = client.getOntologyParents(chebiId);

                if (parents.getListElement().size()==0) {
                        System.out.println("THE END");
                }

                for (OntologyDataItem parent : parents.getListElement()) {
                        if (!parent.isCyclicRelationship()) {
                                System.out.println(parent.getChebiName());
                                traverseOntologyParents(client, parent.getChebiId());
                                break;          //Just find one path to root
                        }
                }
        }
```

Figure 14.9.10 Java code illustrating the navigation of the ChEBI ontology.

3. Ensuring your application can connect to the internet, execute the method `ChebiWebServiceClient.getLiteEntity("benzene", SearchCategory.ALL)`. The result of this execution will be a LiteEntityList, which contains several elements. Traverse the list and print out the names and identifiers of all the returned results. An example Java application is shown in Figure 14.9.8.

4. Get the full entity details for the entity benzene, by executing the method `ChebiWebServiceClient.getCompleteEntity("CHEBI:16716")`. This will return an object of type Entity, from which you can access the full entity data. Navigate the list of database links attached to this entity and for each, print them out. An example Java application is shown in Figure 14.9.9.

5. The ontology can be navigated by repeated execution of the methods getOntologyParents and getOntologyChildren. Write a client application that traverses the ontology parents starting with the entity benzene (CHEBI:16716) up to the root of the ontology, printing out the name of each entity along the path. An example Java application is shown in Figure 14.9.10.

BASIC PROTOCOL 4

DOWNLOADING ChEBI

Many users prefer to download the whole ChEBI database and use it locally. ChEBI is released monthly; therefore, it is important to check that the latest version of the database is used. The entire ChEBI dataset is available for download in several formats.

Necessary Resources

Hardware

A computer with Internet access and 5 Gb of hard disk space available

Software

Internet browser, e.g., Internet Explorer (*http://www.microsoft.com/ie*), Netscape (*http://browser.netscape.com/*), Firefox (*http://www.mozilla.org/firefox/*), or Safari (*http://www.apple.com/safari*)
An RDBMS such as Oracle 9i, MySQL 5 or PostgreSQL
A spreadsheet application such as OpenOffice Calc (*http://www.openoffice.org/*)
A compression/decompression utility that can handle gzip-compressed files (WinZip for Windows, *http://www.winzip.com*; gzip, *http://www.gnu.org/software/gzip/gzip.html, for Linux and other Unix systems*)

1. Go to the ChEBI Downloads page. Downloads may be accessed from the menu on the left-hand side of the main page or directly via this link (*http://www.ebi.ac.uk/chebi/downloadsForward.do*).

   The entire ChEBI dataset is available for download in the following formats:

   i. Flat file, tab delimited. With this format, the data can easily be imported into a spreadsheet application such as OpenOffice Calc, and from there it can be imported into a relational database. It could also be parsed from the flat file and inserted into a custom database structure as required.

   ii. Oracle binary dumps. This is the straightforward Oracle format and it should be imported directly into an Oracle database.

   iii. Generic SQL insert statements, which could be executed on any SQL database. Table creation scripts for use in creating the schema, which corresponds to the SQL insert statements, are provided for MySQL and PostgreSQL databases.

iv. OBO file format for import into the OBO-edit application.

v. SDF file format for visualizing chemical structures and associated data.

Flat file, tab delimited

Data can be imported into a spreadsheet application to be viewed.

2. Click on the Flat file and tab delimited link to download ChEBI in a spreadsheet format. Save the file `compounds.tsv` to your hard disk. Open `compounds.tsv` with your spreadsheet application to view the compound data information.

Relational database management system

ChEBI data may be imported into a relational database management system enabling powerful querying against the data.

3. If you have the Oracle relational database management system available, click on the Oracle binary table dumps link to download ChEBI in Oracle table dump format. Download all files in the `Oracle_dumps` directory, and execute the Oracle `imp` command to import the data as follows:

```
imp database_name/database_password@Instance_name
  PARFILE=import.par
```

4. If you have the MySQL relational database management system, click on the Generic SQL (Structured Query Language) table dumps link to download ChEBI in the form of generic SQL statements. Log into your MySQL command line terminal and execute the `mysql_create tables.sql` script as follows:

```
mysql> source mysql_create_tables.sql
```

5. Unzip the archive `generic_dump.zip` downloaded from the Generic SQL (Structured Query Language) table dumps link on the ChEBI Downloads page. Import the `compounds.sql` file into the database by using the command

```
mysql> source compounds.sql
```

   Import all the files contained in the zip file into the database by replacing the file name in each case, using the command above as an example. For example to import the names table, replace the `compounds.sql` file with `names.sql` as follows:

```
mysql> source names.sql
```

6. If you have the PostgreSQL relational database management systems installed, click on the Generic SQL (Structured Query Language) table dumps link to download ChEBI in the form of generic SQL statements. Log into your PostgreSQL command line terminal and execute the `pgsql_create_tables.sql` script as follows:

```
postgres=# \i pgsql_create_tables.sql
```

7. Unzip the archive `generic_dump.zip` downloaded from the Generic SQL (Structured Query Language) table dumps link on the ChEBI Downloads page. Import the `compounds.sql` file into the database by using the command

```
postgres=# \i compounds.sql
```

   Import all the files contained in the zip file into the database by replacing the file name in each case, using the command above as an example. For example to import the names table replace the `compounds.sql` file with `names.sql` as follows:

```
postgres=# \i names.sql
```

8. Once you have imported the ChEBI data into your RDBMS, you can execute SQL queries against the database and extract the relevant information in which you are interested. Refer to the figure (found on the ChEBI Developer Manual page) for an illustration of the ChEBI domain model in which the data is stored (*http://www.ebi.ac.uk/chebi/developerManualForward.do*).

   *The **Compound** table is the main entry point into the data, referenced by all the other data items. Additionally, it stores the ChEBI recommended name and definition. The Compound table also contains a reference to itself, which is used when duplicate entities within the database are merged.*

   *The **DatabaseAccession** table contains manually annotated references to other databases, such as database links and registry numbers.*

   *The **CompoundName** table contains various types of names such as systematic names, synonyms, and brand names.*

   *The **ChemicalData** table contains formulae and additional chemical data such as charge and mass.*

   *The **Comments** table contains various comments, which may be associated with items in the database.*

   *The **Reference** table contains the automatically generated cross-references to other databases, which are displayed on the Automatic Xrefs tab on the ChEBI Web site.*

   *The **Structure** table contains chemical structures in Molfile, InChI, InChIKey, and SMILES formats. Most entities that have a chemical structure will have one Molfile structure selected as the default. The ID of this default structure will then be present in the **DefaultStructure** table. The IDs of the InChI, InChIKey, and SMILES structures are present in the **AutogenStructure** table as these are automatically generated from the Molfile structure.*

   *The **OntologyModel** describes all the ontologies stored in ChEBI.*

   *Relationships within the ChEBI ontology are represented in the **Relation** table as a directed association between two vertices (represented in the **Vertice** table). Vertices are, in turn, then linked to entries in the Compound table.*

9. To obtain a list of all KEGG accession numbers contained in the ChEBI database along with the name of the entity and the primary ChEBI accession to which they are linked, execute the following SQL query:

```
select NVL(com.parent_id, com.id), com.name,
  da.accession_number
from database_accession da, compounds com
where da.compound_id = com.id
and da.type = 'KEGG COMPOUND accession'
and da.status in ('C','E');
```

   The Compound ID forms part of the ChEBI accession, which is the primary identifier that we encourage use of to refer uniquely to a given chemical entity. Once publicly released, we ensure that the ChEBI accession will be maintained and it will continue to refer to that particular entity. However, as ChEBI is a living and actively maintained database, changes to the dataset do occur, and particularly in cases where duplicate entities in the dataset are merged (e.g., when these entities have been loaded from different source databases).

   *All related accessions are maintained in the Compounds table as children of a parent compound. This is implemented by the self-referencing **parent_id** column in the Compound table. The parent_id contains the ID for the compound specifying the main accession of a merged group of compounds.*

This means that when trying to retrieve the compound accession from a data item such as a database accession or compound name, the relevant entry in the Compounds table must also be retrieved and the parent_id field examined. If the parent_id is not empty, then it links to the compound containing the primary identifier for this merged group of entities.

10. To download the ChEBI dataset in OBO ontology format, click on the OBO file link. The OBO file format is defined by the OBO (Open Biomedical Ontologies; *http://www.obofoundry.org/*) group and is described in detail at *http://www.geneontology.org/GO.format.obo-1_2.shtml*. The file may then be opened in the tool OBO-edit available from *http://oboedit.org/?page=download*.

11. To download the ChEBI dataset in SDF format, click on the SDF file link. The SDF file format is defined by the Symyx and is described in detail at *http://www.symyx.com/downloads/public/ctfile/ctfile.pdf*. The file may be opened with a number of tools such as Bioclipse available from *http://www.bioclipse.net/*.

COMMENTARY

Background Information

ChEBI was originally intended to serve as a controlled vocabulary for a variety of molecular biology databases at the EMBL-EBI and the whole of the biological community. Over time, further data were added to ChEBI, namely molecular structures (in the form of mol files), a chemical ontology, and automatic cross-references (Degtyarenko et al., 2008). Since its first public release (July 21, 2004), ChEBI has grown to represent >17,000 molecular entities, groups, and classes.

ChEBI Maintenance

Automatic initial loading of data

ChEBI systematically combines information on small molecular entities, which are automatically loaded as preliminary data from three main sources: (1) IntEnz database of

Table 14.9.1 Frequently Encountered Problems in ChEBI

Problem	Possible cause	Solution
I can't find the compound using its name	There is no exact text match with the query term	Try using a fragment of the name in combination with wildcards
Why can't I find a known entry in ChEBI even if I use its ChEBI ID as a query?	This ChEBI entry has preliminary status	Send request to the ChEBI team to check this entry
I can't see the structure in the ChEBI entry	Java is not installed	Install Java version 5 or higher
I can't see the structure clearly in the ChEBI entry	The structure of an entity is too complex to be viewed clearly as a static image	Clicking on the Applet button will open an interactive MarvinView applet, which allows a structure to be manipulated. Double-clicking on the applet itself or selecting Window in the drop-down menu opens this as a new window, which can be resized, allowing the structure to be viewed at a higher magnification. Other options in the drop-down menu allow different representations of the structure to be viewed, rotated, etc. Clicking on Image restores the original image view.
When I click Search on the Advanced Search page the response is that I have not submitted any parameters although I have drawn a chemical structure	Javascript is not enabled in your browser	Enable Javascript in your browser

enzymes (EMBL-EBI), (2) KEGG COMPOUND database, and (3) MSDchem database of ligands (also EMBL-EBI).

Preliminary entries are not publicly searchable until they have been manually annotated and checked; however, they may be directly accessed if the identifier is known, or browsed if they are linked to the ontology. They are clearly indicated as preliminary entries in the interface.

Manual annotation

Each preliminary entity is then manually checked and annotated. A unique and unambiguous name is selected as the recommended ChEBI name, the structure is created or checked, an IUPAC name is assigned, and relevant synonyms and database links are annotated.

A number of subsidiary freely accessible sources are manually annotated and integrated, such as ChemIDplus (*http://chem.sis.nlm.nih.gov/chemidplus/*), the NIST Chemistry WebBook (*http://webbook.nist.gov/*), KEGG DRUG (*http://www.genome.ad.jp/kegg/drug/*), and DrugBank (*UNIT 14.4*; *http://www.drugbank.ca/*).

User requests

Users of ChEBI are encouraged to place requests for additions to the dataset via SourceForge (*http://sourceforge.net/projects/chebi/*).

Critical Parameters and Troubleshooting

Table 14.9.1 summarizes some "frequently encountered problems" with suggested solutions.

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Literature Cited

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Degtyarenko, K., Ennis, M., and Garavelli, J. 2007. "Good annotation practice" for chemical data in biology. *In Silico Biol.* 7:S1, 06.

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Steinbeck, C., Hoppe, C., Kuhn, S., Floris, M., Guha, R., and Willighagen, E.L. 2006. Recent developments of the Chemistry Development Kit (CDK): An open-source java library for chemo- and bioinformatics. *Curr. Pharm. Des.* 12:2111-2120.

Key References

Degtyarenko et al., 2008. See above.
Explains main principles of ChEBI. This paper should be used to cite ChEBI.

Kochev et al., 2003. See above.
An excellent introduction to the principles of substructure and structure similarity search.

Internet Resources

http://www.ebi.ac.uk/chebi/
The ChEBI home page.

http://www.ebi.ac.uk/chebi/faqForward.do
ChEBI Frequently Asked Questions.

http://www.ebi.ac.uk/chebi/userManualForward.do
ChEBI User Manual.

http://www.ebi.ac.uk/chebi/tutorialForward.do
ChEBI Tutorials.

http://www.ebi.ac.uk/chebi/annotationManualForward.do
ChEBI Annotation Manual. This manual is designed to enable an annotator (curator) to follow the sequence of steps involved in checking and amending entries in ChEBI.

http://www.ebi.ac.uk/chebi/downloadsForward.do
ChEBI Downloads. Contains the latest ChEBI release in several formats.

http://sourceforge.net/projects/chebi/
ChEBI project at SourceForge.

http://cdk.sourceforge.net
The Chemistry Development Kit project at SourceForge.

http://www.chemaxon.com/marvin/
ChemAxon Marvin documentation page.